Mechanisms of insulin resistance in obese pregnant women: potential therapeutic interventions

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Statement of Contribution

Rahat Maitland arranged sample coordination from participating UK Pregnancies Better Eating and Activity Trial (UPBEAT) sites with analysis carried out by Dr

Lynne Cherry, Elaine Butler and Rahat Maitland at the British Heart Foundation Laboratory (University of Glasgow).

Dr Maitland developed the Improving Glycaemic Profiles in Obese Pregnancies (IGPOP) protocol and study with Dr Suzanne Barr, in collaboration with Professor Lucilla Poston (King's College London, KCL), Drs Ricardo Rueda, Barbara Marriage and Christina Sherry (Abbott Nutrition, Spain and USA) and Dr Helen Murphy (University of Cambridge). During a period of maternity leave for Rahat Maitland, Dr Nashita Patel participated in all aspects of study delivery, data collection and analysis. Erini Platsa (research midwife) together with staff of the clinical research unit (CRF) at St.Thomas' Hospital (London) provided additional support.

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ABSTRACT

Maternal obesity is associated with adverse pregnancy outcomes, especially the development of gestational diabetes mellitus (GDM), with associated risks for mother and infant. Improved understanding of glucose intolerance in obese women and better prediction and prevention of GDM is key to improving the health of mother and her child.

This thesis reports two related projects. The first explored mechanisms of insulin resistance and prediction of GDM in obese pregnant women participating in the pilot study for the UK Pregnancies Better Eating and Activity Trial (UPBEAT), a lifestyle intervention RCT. The second investigated the potential of a dietary intervention to improve glycaemic profiles in this high-risk group.

Following an 8 week dietary and physical activity intervention, a panel of biomarkers associated with obesity and insulin resistance were measured in 117 women in the pilot trial. At 27^{+0}-28^{+6} weeks' no difference was observed between the intervention and control arms but at 34^{+0}-35^{+6} weeks', significant reductions in plasma visfatin, cholesterol and LDL cholesterol were observed. Analysis by GDM status, confirmed greater concentrations of fructosamine, AST and insulin and lower plasma leptin and adiponectin in women who developed GDM. An algorithm based on clinical factors alone (age, parity, ethnicity and blood pressure at 15^{+0}-18^{+6} weeks' gestation) showed predictive potential which increased significantly with the addition of plasma adiponectin measured at 15^{+0}-18^{+6} weeks.

In obese pregnant women without GDM (n=16,) the effect of a slow-digesting low glycaemic index (SD-LGI) supplement drink was evaluated at 24^{+0}-28^{+6} weeks' gestation. Linear regression analysis with mixed modelling (LMM) showed a significant reduction in glycaemia over the 24 hour period following consumption of the test compared to the control supplement and habitual diet. Fasting and nocturnal glucose concentrations were also significantly improved

In summary biomarkers associated with insulin resistance were identified as potential targets for lifestyle interventions aimed at reducing GDM in obese women. A prediction model for GDM identified those at greatest risk and pending validation

in the UPBEAT RCT may have the potential for translation into clinical care. Extending the role of interventions further, multiple improvements in parameters of glycaemic control were demonstrated using a SD-LGI nutritional supplement.

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Abbreviations

ADA	American Diabetes Association
ALSPAC	Avon Longitudinal Study of Parents and Children
ALT	Alanine transaminase
AUC	Area under the curve
BMI	Body mass index
BW	Birth weight

CRP	C-reactive protein
CBG	Capillary blood glucose
CGMS	Continuous glucose monitoring sensors
CMACE	Centre for Maternal and Child Enquiries
CV	Cardiovascular
CVD	Cardiovascular disease
DEXA	Duel energy x-ray absorptiometry
FBG	Fasting blood glucose
FFA	Free fatty acids
GA	Gestational age
GCT	Glucose challenge test
GDM	Gestational diabetes
GGT	Gamma-glutamyl transferase
GI	Glycaemic index
GOx	Glucose oxidase
GTT	Glucose tolerance test
GWG	Gestational weight gain
HbA1c	Haemoglobin A1c (glycated haemoglobin)
HAPO	The hyperglycaemia and adverse pregnancy outcome study
HGI	High glycaemic index
HSE	Health Service for England
HOMA-IR	Homeostatic model assessment of insulin resistance
IADPSG	International Association for the Study of Diabetes in Pregnancy Groups
IGT	Impaired glucose tolerance

IOM	Institute of Medicine
ISF	Interstitial fluid
ISO	International Organization Standard
IVGTT	Intravenous glucose tolerance test
LDL	Low density lipoprotein
LGI	Low glycaemic index
LPL	Lipoprotein lipase
LGA	Large for gestational age
LMM	Linear mixed model
LNP	Lean non-pregnant
LP	Lean pregnant
MTT	Meal tolerance test
MUFA	Monounsaturated fatty acid
NEFA	Non-esterified fatty acid
NGT	Normal glucose tolerance
NSP	Non-starch polysaccharides
OGTT	Oral glucose tolerance test
ONP	Obese non-pregnant
OP	Obese pregnant
PA	Physical activity
PPG	Postprandial glucose
PET	Pre-eclampsia
PUFA	Polyunsaturated fatty acid
RCOG	Royal College of Obstetricians and Gynaecologists
RCT	Randomised controlled trial

RMW	Research midwife
SBP	Systolic blood pressure
SD-LGI	Slow digesting-low glycaemic index
SF	Skinfolds
SFA	Saturated fatty acids
SGA	Small for gestational age
SMBG	Self-monitoring of blood glucose
T1DM	Type one diabetes
T2DM	Type two diabetes
TG	Triglycerides
VLDL	Very-low density lipoprotein
WAT	White adipose tissue
WHO	World Health Organisation
WMD	Weighted mean difference

1 INTRODUCTION

1.1 Current trends in obesity

The worldwide prevalence of obesity has rapidly increased over the past decade with over 1.1 billion adults and 10% of children classified as overweight or obese, in the most recent review by Stevens et al., representing a doubling from 6.4% to 12% (Stevens, Singh et al. 2012). Obesity is now considered to be the seventh most important determinant of adverse health and reduced adult life expectancy globally through an increased risk of cardiovascular disease, type two diabetes (T2DM) and multiple cancers (Whitlock, Lewington et al. 2009, Vineis and Wild 2014).

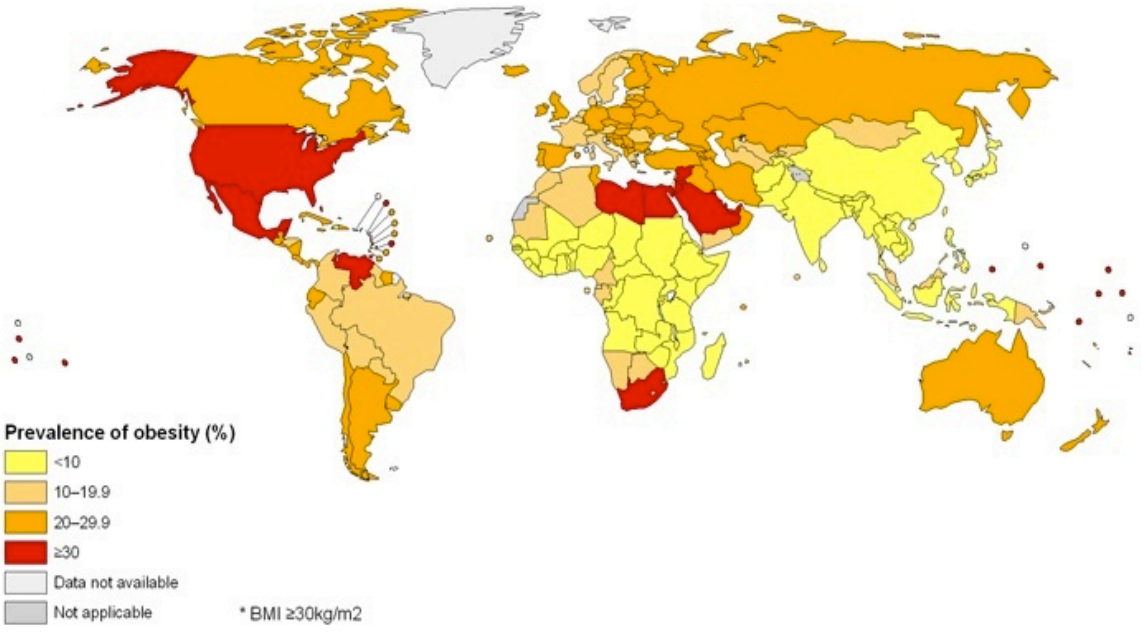


Figure 1 Prevalence of male and female obesity (BMI>30kg/m^2), standardised for age in 2008

Source http://gamapserver.who.int/mapLibrary/Files/Maps/Global_Obesity_BothSexes_2008.png

The universal distribution of obesity remains greatest in developed countries with the lowest prevalence seen in sub-Saharan Africa but a recent shift in trends, parallel to those in Western countries has been observed in regions of high poverty (Popkin and Slining 2013).

Accurate measurement of total body fat remains technically difficult, time consuming and relatively costly therefore obesity is determined and classified in routine clinical practice by measurement of the body mass index (BMI), calculated by the standard formula; weight (kg)/height $(m)^2$. Other techniques such as bioelectrical impedance, measurement of total body water or potassium and duel energy x-ray absorptiometry (DEXA) are reserved for research purposes primarily, with limited availability. Using BMI as a surrogate measure of obesity has multiple advantages but awareness of its limitations is important for translation into clinical medicine. Whilst providing a rapid, inexpensive and replicable estimate of total body fat, measurements are indirect and do not reflect distribution of fat, particularly excess abdominal fat, which is strongly associated with cardiovascular morbidity (Sattar, Tan et al. 1998). Individuals from specified regions, namely South Asia and the South Pacific, have significantly greater risk of adverse health at lower BMI values when compared to White European subjects; nonetheless, the World Health Organisation (WHO) retains the standard international classification system for all, with no adjustment for ethnicity, age or sex (World Health Organisation 2004) (Table 1).

Table 1 WHO classification of weight by body mass index (BMI)

Classification	BMI (kg/m^2)
Normal range	18.5-24.9
Overweight (pre obese)	25.0-29.9
Obese	≥ 30
Class I or moderately obese	30.0-34.9
Class II or severely obese	35.0-39.9
Class III or morbidly obese	≥40.0

The National Health and Nutrition Examination Survey (NHANES) has been examining US trends in adult obesity every two years since 1999, with analysis of a nationally representative sample, aged 20 years and more. The last published survey of 2009-2010 found an age adjusted prevalence of obesity (BMI ≥30kg/m^2) of 35.7% (95% CI [33.8-37.7]) in 8397 men and women across all ethnic groups with similar figures reported in women of reproductive age (20-39 years 31.9%, 95% CI [34.0-37.7])(Flegal, Carroll et al. 2012).

In the United Kingdom, figures for obesity in women of reproductive age have followed a similar trajectory to those in the United States, with a doubling of maternal obesity over the past twenty years observed at the first antenatal visit (Kanagalingam, Forouhi et al. 2005). National data from the Health Survey for England (HSE) showed a rise in obesity rates from 12 to 18.5% in 1993, increasing further to 18.5% in 2006 (HSE 2008). More recently, the latest WHO Global Infobase of statistics, reports an age adjusted prevalence of 26.3% across all ethnic groups in the UK for all women greater than 15 years (WHO 2010).

Maternal obesity is consistently associated with a range of demographic factors. A population based review of pregnancies in England by Heslehurst et al., including 620,000 women with a 1st trimester obesity prevalence of 16%, reported positive associations with age, parity and black ethnicity (Heslehurst, Rankin et al. 2010). Obese women were more likely to be from socially deprived backgrounds with the greatest levels of unemployment observed in women with morbid obesity (BMI $\geq$ 40 kg/m^2).

1.1.1 Gestational weight gain, BMI and anthropometry

Gestational weight gain (GWG) is essential to support the growth of the developing fetus however the optimal amount of gain continues to be re-examined and indeed questions remain as to whether GWG should be the primary measure and targeted modifiable factor, in the management of pregnant women, to reduce disease burden.

Table 2 Institute of Medicine recommendations for total weight gain in pregnancy by pre-pregnancy BMI

Pre-pregnancy BMI (kg/m^2)	**Total weight gain (kg)**	**Rate of weight gain (kg/week)* (2nd & 3rd trimester)**
Underweight (<18.5)	12.5-18	0.51 (0.44-0.58)
Normal (18.5-24.9)	11.5-16	0.42 (0.35-0.50)
Overweight (25.0-29.9)	7-11.5	0.28 (0.23-0.33)
Obese (≥30)	5-9	0.22 (0.17-0.27)

*Calculations assume a first-trimester weight gain of 0.5-2.0 kg

The 2009 revision of the Institute of Medicine (IOM) guidelines on weight gain in pregnancy advise a stricter reduction in GWG in an attempt to minimize adverse

pregnancy outcomes for both mother and offspring and reduce the complications of post partum weight retention, childhood obesity and metabolic dysfunction (Table 2) (Rasmussen and Yaktine 2009).

The variation and degree of GWG is directly related to the number of fetuses per pregnancy (singleton pregnancy 10-16.7kg, twin pregnancy 15-22kg, triplet pregnancy 20.5-23kg) and appears to be influenced by ethnicity and socio-economic class (Rasmussen and Yaktine 2009, Bowers, Laughon et al. 2013). Primiparous women have a tendency for larger GWG in comparison to multiparous women however a recent study by Rasmussen et al., examining almost 3,000 women who participated in a national US cohort study, supports pre-existing data that GWG is inversely related to pre-pregnancy BMI, irrespective of parity (Lan-Pidhainy, Nohr et al. 2013). In the primiparous group (n=822) mean GWG within each BMI category was as follows: BMI<25 17.3 ±5.9kg, BMI 25-29.9 16.4 ±6.9kg and BMI ≥30 12.1 ±7.8kg.

Women with GWG above recommend targets not only have greater risk of obstetric complications including LGA but have been repeatedly shown to have a greater lifetime risk of obesity and metabolic disease (Nelson, Matthews et al. 2010, Fraser, Tilling et al. 2011, Lan-Pidhainy, Nohr et al. 2013). Limitations of this approach include self-reporting of early or pre-pregnancy weight routinely documented at the initial antenatal visit together with the poorly defined composition of total GWG in terms of essential physiological adaptations including placental, blood and fetal weight in contrast to excess adipose accretion.

Debate remains as to the best method of measuring obesity and longitudinal changes in pregnant women which are relevant in research settings and applicable to clinical practice. Aside from gold standard techniques with limited availability including DEXA and Body Plethysmography, BMI, GWG and maternal anthropometry are all surrogate markers of excess adipose tissue. Guidelines published on GWG acknowledge the high inter-person variability and difficulties in determining the changing composition of GWG during pregnancy as the fetus develops suggesting that GWG is a poor measure of accretion of fat mass. The frequently quoted ‘Y-Y paradox’ of two men with the same BMI and very different body composition described in 2004 clearly highlights limitations of using BMI as a surrogate marker

of adiposity; both had a BMI of 22.3kg/m^2 but DEXA scanning revealed a significant difference in total body fat of 9.1% and 21.2% (Yajnik and Yudkin 2004). Adipose tissue is now recognised as an important endocrine organ with varied clinical risk attributed to the pattern of fat distribution (Lee, Wu et al. 2013). BMI does not reflect body fat distribution and since the developing fetus and normal physiological increase in fluids are the main contributors of GWG, it remains valid only in pre-pregnancy or the early stages (Figure 2).

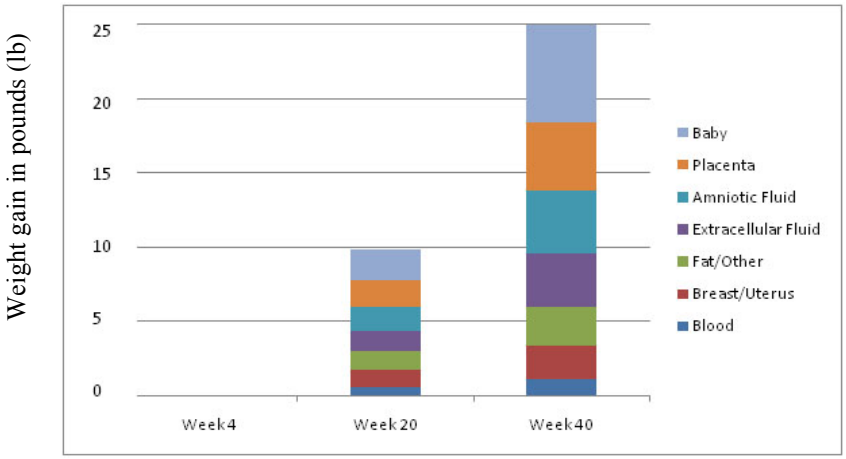


Figure 2 Estimated composition of Maternal GWG (Rasmussen and Yaktine 2009)

Maternal anthropometry, measured by skin fold thickness using validated callipers at selected sites, can be used as a surrogate maker of adiposity in pregnant women with the benefit of reflecting longitudinal changes during gestation and being easily replicated in clinical practice. In adults, body composition is considered a better predictor of health outcome than body weight alone (Thibault, Genton et al. 2012). Ongoing collaborative work by international experts participating in the European EarlyNutrition Academy and EarlyNutrition Project (www.project-earlynutrition.eu) continues to determine the optimal methods of measuring neonatal/offspring adiposity to enable prediction of disease risk later in life (Ward, Poston et al. 2013).

1.2 Adverse pregnancy outcomes in obese pregnant women

A large body of supporting data has identified numerous adverse health outcomes to mother and fetus during an obese pregnancy which describe a linear continuous relationship with increasing BMI (Sebire, Jolly et al. 2001, Chu, Callaghan et al. 2007, Athukorala, Rumbold et al. 2010, Nelson, Matthews et al. 2010). These apply

to complications during pregnancy, labour and the postpartum period with consequences to maternal and offspring health in later life.

In addition to disease burden, the resource implications and logistics of providing more intensive obstetric and lifetime healthcare to obese women and their offspring, continues to be an important issue as shown in a case controlled study by Galtier-Dereure et al. comparing the duration of hospitalisation in women with a pregravid BMI>25kg/m^2 (Galtier-Dereure, Boegner et al. 2000). Overweight women had a longer duration of hospitalisation (by 3.9 and 6.2 fold for day and night periods respectively) and spent an average of 4.43 more days in hospital compared to lean matched controls (BMI 18-25kg/m^2) when data for pre and post pregnancy care were combined. The most recent cost analysis of obstetric care for obese women in Scotland found similar results with more frequent and prolonged hospital admissions, equating to a mean additional admission cost of £202.46 compared to lean women following analyses adjusted for maternal age, parity, smoking status and deprivation (mean additional cost £202.46, 95% CI [178.61-226.31], $p<0.001$) (Denison, Norwood et al. 2014).

1.2.1 Maternal adverse outcomes

Obstetric Complications

Several recent large cohorts, systematic reviews and meta-analyses have provided estimated risks for pregnancy complications following maternal obesity.

Pre-gravid obesity is associated with sub-fertility and increased rate of both incident and recurrent miscarriage (OR for recurrent miscarriage 4.68, 95% CI [1.21 to 18.13]) (Metwally, Ong et al. 2008). The risk of the most frequently observed congenital anomalies are greater including spina bifida and cardiac malformations (OR 2.24, 95% CI [1.86 to 2.69] and OR 1.3, 95% CI [1.12-1.51] respectively) (Stothard, Tennant et al. 2009) whilst gestational hypertension (RR 3.19, 95% CI [2.36 to 4.30]) (Athukorala, Rumbold et al. 2010, Stuebe, Landon et al. 2012), pre-eclampsia (OR 2.14, 95% CI [1.85 to 2.47]) (Sebire, Jolly et al. 2001), and venous

thromboembolism are all more prevalent (Centre for Maternal and Child Enquiries (CMACE) 2010).

Obstetric complications are significantly more common in obese women, for example preterm birth, induction of labour, elective and emergency caesarean section, instrumental delivery and postpartum haemorrhage (Sebire, Jolly et al. 2001, Heslehurst, Simpson et al. 2008, Athukorala, Rumbold et al. 2010, Nelson, Matthews et al. 2010, Denison, Norwood et al. 2014). The administration of anaesthesia, whether regional or general, poses greater hazards in this population and the risks of standard post-operative complications such as respiratory ventilation, wound infection and length of stay are all increased.

Abnormal Glucose Homeostasis

Pregnancy in healthy subjects is a period of metabolic stress characterised by hepatic and peripheral insulin resistance (IR), with co-existing alterations in lipid and protein metabolism aimed at meeting the energy requirements of the developing fetus (Nelson, Matthews et al. 2010, Catalano and Hauguel-De Mouzon 2011). Obese women, with an already heightened pregravid risk of T2DM, have a 2 to 4 fold increased risk of developing GDM (Table 3). A recent Scottish population study of 124, 280 deliveries reported significantly greater odds of developing GDM for obese and morbidly obese women when compared to healthy weight women, when adjusted for maternal age, social deprivation and smoking following multiple regression (obese OR 11.90, 95% CI [7.54-18.79], morbidly obese OR 67.40, 95% CI [37.84-120.03]) (Denison, Norwood et al. 2014). The actual numbers of GDM cases in this national database cohort were very low however affecting only 0.9% of obese (205/21,634) and 3% of morbidly obese (87/2720) women. Chu et al. analysed 20 studies in their meta-analysis, eight of which were conducted in the United States and the remaining 12 from Europe, Australasia, the Middle East, and Nova Scotia including subjects from the Native Cree Indians known to have a very high risk of developing T2DM (Zimmet 2003).

The influence of ethnicity on the development of GDM is well established with women from certain ethnic groups, particularly from South Asia, Latin America,

South Pacific islands and Native Indian groups, having substantially greater risk of developing GDM than white European women of equivalent BMI (Ferrara 2007).

Table 3 Summary of four studies investigating the association of maternal BMI and development of GDM

	Maternal BMI category versus normal (odds ratio [95% CI])		
	Overweight	**Obese**	**BMI (>40kg/m^2)**
Sebire (2001)	1.68 (1.56-1.84)	3.6 (3.25-3.98)	-
Chu (2007)*	2.14 (1.82-2.53)	3.56 (3.05-4.21)	8.56 (5.07-16.04)
Athukorala (2010)	1.21 (0.66-2.21)	2.10 (1.17-3.79)	-
Denison (2014)	3.39 (2.30-4.99)	11.90 (7.54-18.79)	67.40 (37.84-120.03)

* Meta-analysis of 20 studies

The obese groups in the two additional studies detailed in Table 3 (Sebire et al. [n=287, 213]) and Athukorala et al. [n= 1611]) were composed of predominantly Caucasian subjects (71.4% and 96% respectively). Other co-founding factors for the development of GDM are similar to those for obesity and include maternal age, parity, ethnicity, family history of diabetes and low socio-economic class (Heslehurst, Rankin et al. 2010).

Until recently, there has been no internationally agreed diagnosis for GDM and clinical practice has varied considerably. Following the seminal Hyperglycaemia and Adverse Pregnancy Outcome Study (HAPO), (The HAPO Study Cooperative Research Group 2008), the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) published new diagnostic criteria which have been recommended by the American Diabetes Association (ADA) (IADPSG 2010) and more recently the WHO (WHO 2013). Table 4 illustrates the different glucose concentrations required for a diagnosis of GDM prior to the adoption of the 2010 IADPSG guidelines.

Controversies remain as to the best approach to diagnosing GDM since the new glucose thresholds are lower than any previous cut offs, thereby identifying significantly more cases of GDM and changing clinical care for substantially more women. Although the HAPO study was an observational prospective study, the authors demonstrated a clear, continuous, linear relationship between glucose

concentration and a composite primary endpoint of adverse events (birth weight ≥90th centile, primary cesarean delivery, neonatal hypoglycemia, and cord-blood serum C-peptide ≥90th centile). At the time of the HAPO study it was considered ethical to not treat GDM therefore the results provide insight into the adverse effects of non-treatment of varying glucose concentrations below those generally accepted as indicative of overt diabetes.

Table 4 Comparison of criteria for diagnosis of GDM

Test	**IADPSG (any 1 of)**	**ADA* (at least 2 of)**	**WHO (2006) *IFG/IGT** (any 1 of)**
Fasting glucose (mmol/l)	≥5.1	≥5.3	≥6.1
1h-glucose (mmol/l)	≥10.0	≥10.0	-
2h-glucose (mmol/l)	≥8.5	≥8.6	≥7.8

*The ADA and WHO have since endorsed the IADPSG recommendations

**IFG/IGT: Impaired fasting glucose/impaired glucose tolerance

O'Sullivan et al. assessed the impact of the new IADPSG guidelines on maternal outcomes in 5500 women of predominantly white European descent participating in the Atlantic Diabetes in Pregnancy study (DIP). As anticipated, the prevalence of pregnancy complications was significantly greater in women with IADPSG GDM versus women with normal glucose tolerance (gestational hypertension 13.8% v 7.5% p<0.0001, preeclampsia 6.3% v 4.0% p=0.007, polyhydramnios 3.4% v 0.8% p<0.0001, normal vaginal delivery 51.4% v 57.9% p=0.002 and caesarean section 37.2% v 24.9% p<0.0001) (O'Sullivan, Avalos et al. 2011). A direct comparison of adverse outcomes for GDM diagnosed by IADPSG and WHO 2006 criteria to explore the effects of the lower glucose concentration was not performed but following its introduction, centres have observed a 2-8 fold increase in cases of GDM depending on local ethnicity and obesity (Langer, Umans et al. 2013).

Pregnancy complicated by GDM confers a substantially greater lifetime risk of developing type 2 diabetes (T2DM) (Lauenborg, Hansen et al. 2004). Considering the multiple limitations of attempting to quantify this risk in large meta-analyses, including different diagnostic criteria of GDM and T2DM, wide variations in the duration of post-natal follow up and multiple confounding factors including

ethnicity, Bellamy et al. reported an overall relative risk of 7.43 (RR 7·43, 95% CI [4·79–11·51]) (Bellamy, Casas et al. 2009). Women with GDM have progressive changes with advancing gestation, to measures of insulin sensitivity, insulin resistance and beta cell function, reflecting a worsening degree of glucose intolerance. Xiang et al., followed up women 5 years from their last pregnancy, with and without a previous history of GDM over a median of 4 years, measuring insulin resistance and changes in adiposity using DEXA (Xiang, Takayanagi et al. 2013). Women with GDM not only had significantly more impaired fasting glycaemia (IFG) and impaired glucose tolerance (IGT) at enrolment (baseline IFG: 35.5% v 13.4% and IGT: 71.0 v 39.4%, GDM v non-GDM respectively) and four years later but an accelerated rate of decline in glucose tolerance, measured during an intravenous glucose tolerance test (IVGTT).

Pre-pregnancy obesity and GDM are independent predictors of multiple adverse pregnancy outcomes however the presence of both confers additional risk than either one alone. Catalano et al. analysed all women who participated in the HAPO study (n=23,316); 13.7% were classified as obese and 16.1% diagnosed with GDM with 25% of this cohort obese also (Catalano, McIntyre et al. 2012). Increased odds ratios were found for a combination of the two conditions than either alone for six adverse outcomes including cord C-peptide, preeclampsia, birth weight >90th centile and newborn percentage fat, with an almost doubling of effect for selected outcomes. The independent effect of obesity and GDM on adverse outcomes is complex and remains difficult to disentangle due to the physiological interrelationship and causality between the two and study design.

In response to extensive data detailing increased pregnancy complications for obese women, with particular reference to the Centre for Maternal and Child Enquiries (CMACE) 2010 report titled “Maternal obesity in the UK: Findings from a national project,” guidelines have been published in conjunction with the Royal College of Obstetrics and Gynaecology (RCOG) to provide and support alternate clinical pathways for this high risk group (Modder and Fitzsimmons 2010).

1.2.2 Offspring adverse outcomes

Maternal obesity influences growth and development of the fetus. Pre-pregnancy BMI is a strong predictor of mean birth weight, with obese women having 2-5 fold increased risk of delivering an infant who is large for gestational age (defined as >90th centile [LGA]) or macrosomic (>4000g) (Heslehurst, Simpson et al. 2008, Athukorala, Rumbold et al. 2010, Bowers, Laughon et al. 2013), with some evidence to suggest that obesity has a greater influence than GDM on LGA despite the strong interrelationship (Catalano and Ehrenberg 2006).

Observational data indicates a higher risk of pregnancy complications associated with excess GWG for obese women when compared to lean subjects, suggesting a potentially greater influence of pre-pregnancy BMI rather than GWG on adverse outcomes (Nohr, Vaeth et al. 2008). A retrospective analysis of 57,700 women participating in the Danish National Birth Cohort (DNBC) from 1996–2002, demonstrated an inverse relationship between the risk of giving birth to a small for gestational age infant (defined as <10th centile [SGA]) with pre-pregnancy BMI and GWG. Furthermore the opposite effect was observed for LGA whereby obese women or those with excessive GWG had the greatest risk. For all maternal and neonatal outcomes measured (pre-eclampsia, hypertension, GDM caesarean section rate, instrumental delivery, SGA, LGA and low Apgar score at 5min), pre-pregnancy BMI was the strongest predictor although GWG was significant for postpartum weight retention at six months for all categories of BMI (Figure 3).

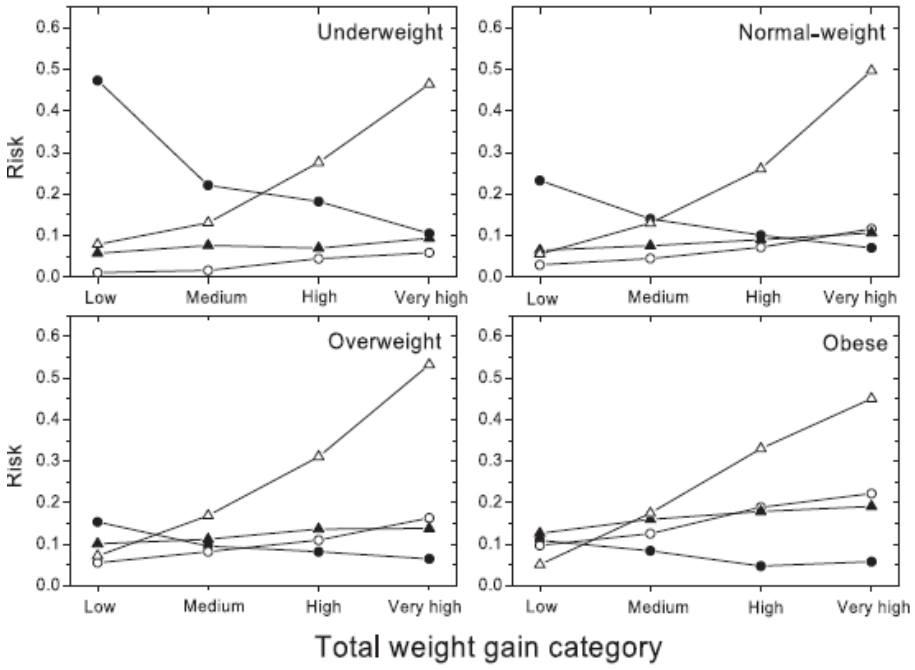


Figure 3 Adjusted absolute risks for SGA (●), LGA (○) emergency CS (▲) and PPWR ≥5kg at 6m (Δ) according to pre-pregnancy BMI and WHO GWG categories (low<10kg, medium 10-15kg, high 16-19kg and very high ≥20kg) (Nohr, Vaeth et al. 2008)

Neonatal macrosomia increases the risk of shoulder dystocia, birth trauma and depression of Apgar scores whilst infants of women with GDM face a significantly higher risk of neonatal hypoglycaemia requiring treatment (Sebire, Jolly et al. 2001). Subsequently, infants are more likely to require admission to intensive care areas, with longer periods of hospitalisation.

Findings from the 2010 CMACE national survey corroborate previous data identifying a greater prevalence of stillbirth and perinatal loss in obese women (Chu, Kim et al. 2007). With a background population rate of 3.9 per 1000 birth, women with a BMI≥35kg/m^2 were almost twice as likely to have a stillbirth in the United Kingdom (8.6 stillbirths per 1000 total births) (Centre for Maternal and Child Enquiries (CMACE) 2010).

Although the differences in birth weight may be considered small and subtle initially, longitudinal cohort studies have confirmed increased associated lifelong risk of obesity and metabolic disease for these children (Fraser, Tilling et al. 2010). In The Early Childhood Longitudinal Study (n=7738), 12% of the children born with

macrosomia represented 36% of 14 year olds who were obese (Cunningham, Kramer et al. 2014).

1.2.3 Long term offspring metabolic sequelae

Multiple large prospective birth cohorts examining the effect of GWG and pre-pregnancy BMI on infant outcomes report strong positive associations with offspring adiposity from childhood, (Oken, Taveras et al. 2007) through to adolescence (Yajnik and Yudkin 2004) and adulthood (Lee, Yuanyuan et al.). Most recently in a meta-analysis of 45 studies of medium to high quality, offspring of obese women had a three-fold increased risk of adult obesity (Yu, Han et al. 2013) associated with a significantly heightened risk of morbidity and excess mortality notably from cardiovascular disease (Reynolds, Allan et al. 2013, Yu, Han et al. 2013). Data for 37,709 mother:infant pairs extracted from the Aberdeen Maternity and Neonatal Databank (AMND) demonstrated that offspring of obese women had increased all cause mortality (hazard ratio 1.35, 95% CI 1.17 to 1.55) including premature mortality secondary to cardiovascular disease (stroke, angina and myocardial infarction aged<55 years) and were more likely to require hospital admission for a cardiovascular event (Reynolds, Allan et al. 2013). While these results were adjusted for many confounding factors (maternal age at birth, offspring sex, socioeconomic status, gestational age at delivery and current age and birth weight), quantifying and assessing the influence of non-modifiable factors including ethnicity and inherited transmission is challenging.

Attempts to identify the risk of offspring obesity and diabetes, following a pregnancy affected by diabetes, over and above that attributable to genetic factors alone, have been made with interesting results.

Dabelea et al. identified 19 families where at least one pregnancy occurred either side of a diagnosis of maternal T2DM. In doing so, they were able to follow up siblings from the same parents who had been exposed to different intrauterine environments but with similar genetic susceptibility (Dabelea, Hanson et al. 2000). When examined at a comparable age, those siblings exposed to maternal diabetes were more likely to develop T2DM themselves (OR 3.7, 95% CI [1.3-11.3], p=0.02) and have a higher BMI than their older siblings (mean difference in BMI 2.6kg/m^2,

95% CI [0.9-4.3], p=0.003]) up to early adulthood. In a similar analysis examining the paternal influence, where only the father had T2DM, no difference was observed between siblings for risk of diabetes or obesity therefore strengthening the association of abnormal maternal glucose tolerance with offspring metabolic outcomes. Appreciably the results may be skewed since the sample was comprised of Native Pima Indians of Arizona, an ethnic group known to have extremely high prevalence of both obesity and T2DM. Nonetheless the importance of the intrauterine environment with reference to abnormal glucose homeostasis, as a determinant off offspring adverse health, independent of genetic risk, is illustrated.

For women with normal pre-pregnancy BMI, excessive GWG also increases the risk of metabolic dysfunction although pre-gravid obesity remains the stronger determinant. The Avon Longitudinal Study of Parents and Children (ALSPAC) is an on-going prospective population-based birth cohort (n=14,541) of mother:infant pairs, delivered between 1991-1992. In a sub study of 6668 pairs, with complete data up to 9 years, offspring of women who exceeded the 2009 IOM recommendations for GWG not only had significant differences in clinical measurements (BMI, waist circumference, SBP, DBP and total fat mass) but positive and negative changes in selected biomarker concentrations (HDL-C, leptin and apolipoprotein A1) associated with cardiovascular disease (Fraser, Tilling et al. 2010).

The positive linear relationship between GWG and offspring adiposity was present for all women up to 14 weeks but stronger for those who gained more than 500g/week. During the 1st trimester, weight gain typically represents an increase in physiologically active maternal fat stores rather than fetal mass. Thereafter from 14-36 weeks gestation, GWG was only associated with infant adiposity for women with accelerated weight gain.

In contrast, the relationship between GWG and adverse biomarker concentrations attenuated towards the null in the first trimester and was only significant from 14-36 weeks. The authors suggest a possible lack of statistical power for the absence of a significant relationship until 14 weeks however the transmission of fat related CV risk may be mediated by alternative pathways related to the location of adipose stores, accrued at different stages of pregnancy, rather than total fat gain. It is likely that the production of adipokines is not uniform since depot-specific hormonal

changes, conferring varied degrees of adverse risk, have been observed for different compartments e.g. visceral, subcutaneous, upper and lower body (Wajchenberg, Giannella-Neto et al. 2002). An independent relationship between GWG and offspring adiposity has now been identified in several different mother-child cohort studies (Poston 2012).

1.3 Potential mechanisms of adverse outcomes in obese pregnancies

1.3.1 Distribution of adipose tissue in lean and obese women

1.3.1.1 Non-Pregnant State

Multiple international collaborations totalling almost 400,000 subjects, including the European Prospective Investigation into Cancer and Nutrition (EPIC) (Pischon, Boeing et al. 2008) and case-controlled INTERHEART (Yusuf, Hawken et al. 2005) studies have consistently found strong associations between measures of central obesity and visceral fat with features of the metabolic syndrome, cardiovascular morbidity and mortality (Schneider, Friedrich et al. 2010). Results from the Emerging Risk Factors Collaboration reported that BMI, waist circumference and waist-to-hip ratio all had comparable strength of association with ischaemic stroke and coronary heart disease suggesting no additional benefit of taking further anthropometry measurements than those already routinely used (Wormser, Kaptoge et al. 2011).

Evidence however continues to demonstrate that visceral fat exhibits increased metabolic and functional activity compared to subcutaneous fat depots, secreting a wide array of pro-inflammatory cytokines, adipokines and increased concentrations of free fatty acids (FFA), all implicated in the causal pathway of the metabolic syndrome (Wajchenberg 2000, Wajchenberg, Giannella-Neto et al. 2002, 2010). In contrast to upper body adiposity, lower body fat stores appear to be associated with a reduced risk of metabolic dysfunction however the relative contributions of upper body subcutaneous and visceral fat to this heightened risk remains undifferentiated (Snijder, Dekker et al. 2004). As a surrogate measure, BMI is unable to distinguish between obesity caused by muscle or fat accumulation, an important limitation

considering the different physiological and potentially pathogenic roles of adipose tissue. Since pregravid maternal BMI has been shown to be a stronger predictor of adverse outcome than GDM and excessive GWG, examining different fat stores in non-pregnant subjects and how these change during pregnancy seems warranted (Catalano and Ehrenberg 2006, Nohr, Vaeth et al. 2008).

1.3.1.2 During Pregnancy

In a small study longitudinal prospective study, Ehrenberg et al. quantified maternal adiposity in lean and obese subjects with and without GDM following calculation of fat mass (FM), lean body mass (LBM) and percentage body fat (BF) using hydrodensitometry corrected for residual lung volume at preconception, in early and late gestation (12-14 and 33-36 weeks' respectively) (Ehrenberg, Huston-Presley et al. 2003). Central adiposity was measured antenatally using waist:thigh ratio and subcutaneous fat determined with serial skinfold measurements at 7 sites (biceps, triceps, subscapular, iliac, costal, mid thigh and lower thigh). Lean women gained significantly more fat mass than obese women during pregnancy (change in percent of BF 3.3% v 0.1%, $p=0.004$) with a preference for peripheral deposition (biceps, triceps, costal, $p<0.05$ for all) compared to central deposition in obese women (suprailiac and subscapular skin folds). Women who developed GDM had similar accretion and distribution of weight gain when lean and obese groups were compared suggesting a stronger influence of pregravid weight rather than impaired glucose tolerance on adipose stores and function.

The hypothesis that different compartments of adipose tissue confer greater risk for metabolic disease later in life whilst others stores may be considered "safer" or even "protective" has been established in the non pregnant state (Yusuf, Hawken et al. 2005, Pischon, Boeing et al. 2008, Schneider, Friedrich et al. 2010) but studies in pregnancy with detailed measures of weight and fat distribution related to clinical outcomes are limited (Yusuf, Hawken et al. 2005, Pischon, Boeing et al. 2008, Schneider, Friedrich et al. 2010).

The relationship and predictive power of longitudinal changes to adipose tissue depots during pregnancy and GDM was recently explored by a Norwegian group (Sommer, Morkrid et al. 2014). Comparing data obtained prospectively between 15-

28 weeks' gestation in 728 women (mean pre-pregnancy BMI 24.5kg/m^2), the mean weekly weight gain was 0.51kg, thus exceeding the IOM 2009 recommendations (0.42kg/week for lean subjects). Total fat mass and truncal adiposity measured using bioelectrical impedance, increased by 0.38 and 0.22kg per week respectively and using modified IADPSG GDM guidelines (1hour glucose not available) a prevalence of 31.5% GDM was recorded. In keeping with previous evidence examining T2DM risk in non-pregnant adults, an increase in central adiposity was strongly associated with GDM (OR 1.31, 95% CI [1.10-1.56]) compared to weight gain (OR 1.23, 95% CI [1.04-1.16]) and mean skinfold measures (OR 1.22, 95% CI [1.02-1.47]) however caution interpreting these results must be used since truncal adiposity is a component of total fat mass and the difference in odds ratios were small. Furthermore truncal fat measures incorporate subcutaneous and visceral fat both considered to have different properties and risk in relation to CVD.

Concordant with extensive literature, women of South Asian heritage were three times more likely to develop GDM than European women when the combined effects of pre-pregnancy BMI and truncal fat gain were included in the regression model (OR 5.9, 95%CI [3.5-10.0] and OR 2.1, 95% CI [1.6-2.8]). The single effect of pre-pregnancy BMI was greater than change in central adiposity (Figure 4) highlighting the importance of targeting pre-conception weight loss as the preferred time to achieve improved clinical outcomes, albeit difficult to attain in practice.

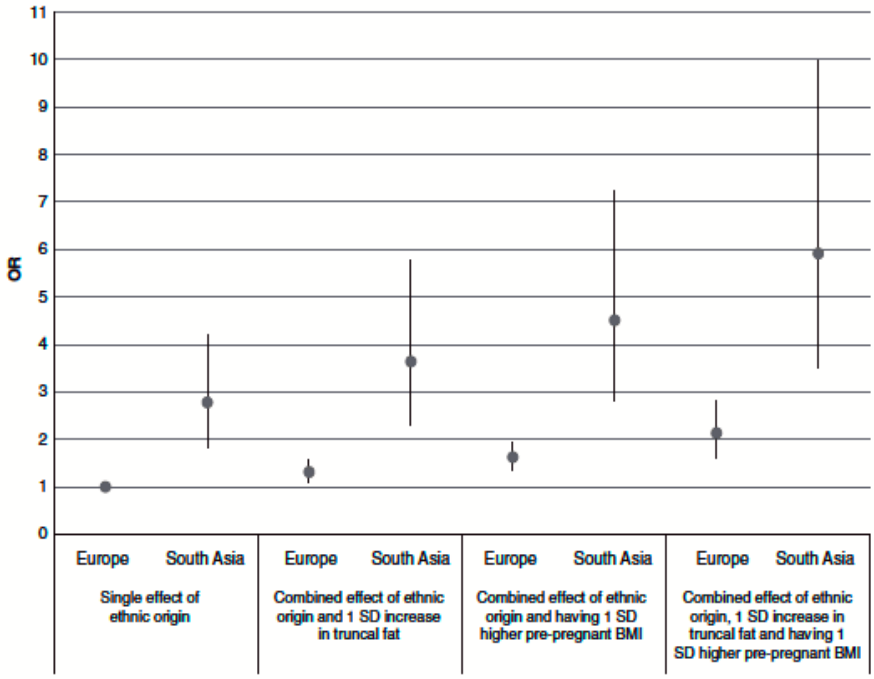


Figure 4 Plot to illustrate the single effect of ethnicity and the combined effects of ethnic origin, 1 s.d. (0.14kg/week) truncal fat gain and 1 s.d. (4.7kg/m^2) higher pre-pregnant BMI for Europeans and South Asians on the risk of GDM. Dots are ORs and lines are 95% CI (Sommer, Morkrid et al. 2014)

1.3.2 Neonatal adiposity and maternal obesity

Differences in measures of neonatal adiposity are apparent from birth following an obese pregnancy and when considering multiple contributory factors including smoking status, excess GWG, sex, maternal age, parity and gestational age at birth, maternal obesity remains the strongest predictor of percentage body fat and fat mass (Waters, Huston-Presley et al. 2012, Dello Russo, Ahrens et al. 2013, Ensenauer, Chmitorz et al. 2013).

The effects of maternal obesity and GDM on fetal outcome are independent and additive (Catalano and Ehrenberg 2006, Simmons 2011) however the individual influences on fetal adiposity remains difficult to separate and complex to assess. In the 1950's Jorgen Pedersen postulated that fetal overgrowth was related to an increase in transplacental glucose transfer secondary to maternal hyperglycaemia, resulting in fetal islet cell hypertrophy and hyperinsulinaemia (Pedersen 1952). There is work to suggest that infants born to women with abnormal glucose tolerance have greater fat mass but not birth weight compared with offspring of healthy women when corrected for gestational age (Catalano, Thomas et al. 2003) (Table 5).

In this study by Catalano et al., direct comparison of BMI between women with and without GDM was not performed therefore BMI remains a significant confounding factor. Nonetheless, women in the GDM group were significantly heavier prior to conception (81.0 v 65.5kg, p=0.001) and following adjustment for parental height and weight the differences observed in fetal adiposity between the GDM and non-GDM groups remained.

Table 5 Offspring body composition (total body electrical conductivity TOBEC) and anthropometry in infants of women with and without GDM (Catalano, Thomas et al. 2003)

Variable**	GDM (n=195)	NGT (n=220)	P value*
Weight (g)	3398 ± 550	3337 ± 549	0.26
Fat-free mass (g)	2962 ± 405	2975 ± 408	0.74
Fat mass	436 ± 206	362 ± 198	0.0002
Body fat (%)	12.4 ± 4.6	10.4 ± 4.6	0.0001
Triceps (mm)	4.7 ± 1.1	4.2 ± 1.0	0.0001
Subscapular (mm)	5.4 ± 1.4	4.6 ± 1.2	0.0001
Flank (mm)	4.2 ± 1.2	3.8 ± 1.0	0.0001
Thigh (mm)	6.0 ± 1.4	5.4 ± 1.5	0.0001
Abdomen (mm)	3.5 ± 0.9	3.0 ± 0.8	0.0001

*Following adjustment for gestational age, maternal pregravid weight, weight at last antenatal visit, race, smoking status, and maternal and paternal height **values are given as mean ± SD

The HAPO study showed a continuous linear relationship between maternal glucose, cord C-peptide and birth weight (The HAPO Study Cooperative Research Group 2008) lending support to the Pedersen hypothesis and extending this further. Data from the ALSPAC cohort suggests that any degree of glucose intolerance during pregnancy including glycosuria (++ on urinalysis equating to 250mg/ml, present on minimum 2 antenatal visits), which may be transient, has an adverse effect on offspring adiposity at 9-11 years (Lawlor, Fraser et al. 2010). Historically, macrosomia was considered a consequence of unrecognised maternal hyperglycaemia but in the context of optimal glycaemic control, confirmed with CGMS, women with all types of diabetes in pregnancy (pre-existing T1 and T2DM and GDM), continue to have a threefold greater risk of having a large infant

compared to the background population (Evers, de Valk et al. 2002, Langer, Yogev et al. 2005, Murphy, Rayman et al. 2008).

For obese women, the risk of macrosomia is a function of pregravid obesity with additional risk conferred by even relatively small changes in glucose tolerance, which may not meet the threshold for a formal diagnosis of GDM (Yogev, Ben-Haroush et al. 2004). Langer et al. showed that obese women with well-controlled GDM managed with diet treatment alone still have a 50% greater chance of having a large infant compared to lean women with GDM on similar treatment. The risk of macrosomia reduced only for obese women with GDM requiring insulin therapy to optimise glycaemia (Langer, Yogev et al. 2005). The data suggests that the insulin-mediated effects of reducing excess fetal growth are related to other metabolic pathways in addition to glucose, in particular lipid metabolism.

1.3.3 Metabolic adaptations in obese pregnant women

Maternal obesity is associated with dysregulation of metabolic, vascular and inflammatory pathways (Ramsay, Ferrell et al. 2002, Stewart, Freeman et al. 2007, Meyer, Stewart et al. 2013) frequently implicated in the pathogenesis of GDM and T2DM (Sattar, Wannamethee et al. 2008, Savvidou, Nelson et al. 2010, Ferreira, Rezende et al. 2011).

Lipid metabolism

During pregnancy, hyperlipidaemia occurs with a 2-4 fold increase in triglyceride (TG) concentration with a concomitant progressive rise in total cholesterol (Nelson, Matthews et al. 2010). In addition, concentrations of lipids strongly associated with adverse cardiovascular health, very-low density (VLDL) and low-density lipoprotein (LDL), increase from early second trimester by a magnitude of 3 to 4, paralleled by lesser rise in the more cardio-protective high-density lipoprotein (HDL) (Lain and Catalano 2007, Nelson, Matthews et al. 2010).

Early gestation promotes greater storage of free fatty acids (FFAs) in adipose tissue contributing to the required anabolic environment but from mid-late gestation, mobilisation of FFAs from increased lipolysis mediated by LPL occurs in response to greater energy demands not adequately met by glucose (Huda, Sattar et al. 2009).

A 50-80% increase in basal fat oxidation and subsequent release of FFAs plus rising concentrations of circulating triglycerides, contributes to overall insulin resistance; a response that is exaggerated in obese women.

The ability of insulin to suppress lipolysis in late pregnancy is diminished in all women but to a greater degree in obesity, adding to the overall pool of circulating FFAs that cross the placenta and enter the fetal adipocyte (Catalano and Hauguel-De Mouzon 2011). In the mature adipocyte, TGs are the primary component of intracellular lipid stores, derived from esterification of FFAs and non-lipid precursors, precursors, for example glucose derived from carbohydrate metabolism and lipolysis. lipolysis. Maternal sources of glucose, including those from dietary sources, are transported through the fetal portal circulation to provide substrate for de novo lipid synthesis within adipocytes (

Figure 5) (Catalano and Hauguel-De Mouzon 2011).

Lipid dysregulation and increased deposition of visceral fat in obese pregnant women contributes to a spectrum of metabolic outcomes which share causal pathways including GDM (Lain and Catalano 2007), intrahepatic cholestasis of pregnancy (Martineau, Raker et al. 2014) and pre-eclampsia (Vrijkotte, Krukziener N Fau - Hutten et al. 2012).

Ramsay et al. analysed a detailed panel of biomarkers reflecting lipid metabolism and inflammatory processes together with measures of in-vivo endothelial function, obtained using doppler imaging, in lean and obese women in the 3rd trimester. Obese women had significantly greater concentrations of TG, leptin, fasting plasma insulin, Interleukin-6 and C-reactive protein with lower concentrations of HDL in keeping with a more atherogenic profile (Ramsay, Ferrell et al. 2002) A similar pattern in lipid metabolites was observed in a more recent study by Meyer et al. who sampled obese women longitudinally from 12-36 weeks' gestation (Meyer, Stewart et al. 2013). Interestingly, in the study by Ramsay et al., fasting plasma insulin concentrations in obese women were more than double those in lean counterparts, without any alteration in glucose tolerance; glycated haemoglobin (HbA1c) was equivalent for both groups of women (mean fasting plasma insulin 14.2mU/l [IQ

range 11.3–27] versus 6.15mU/l [IQ range 4.47–9.5], p<0.0001 and HbA1C 4.5% v 4.4%, p=0.89 for obese and lean respectively) (Ramsay, Ferrell et al. 2002).

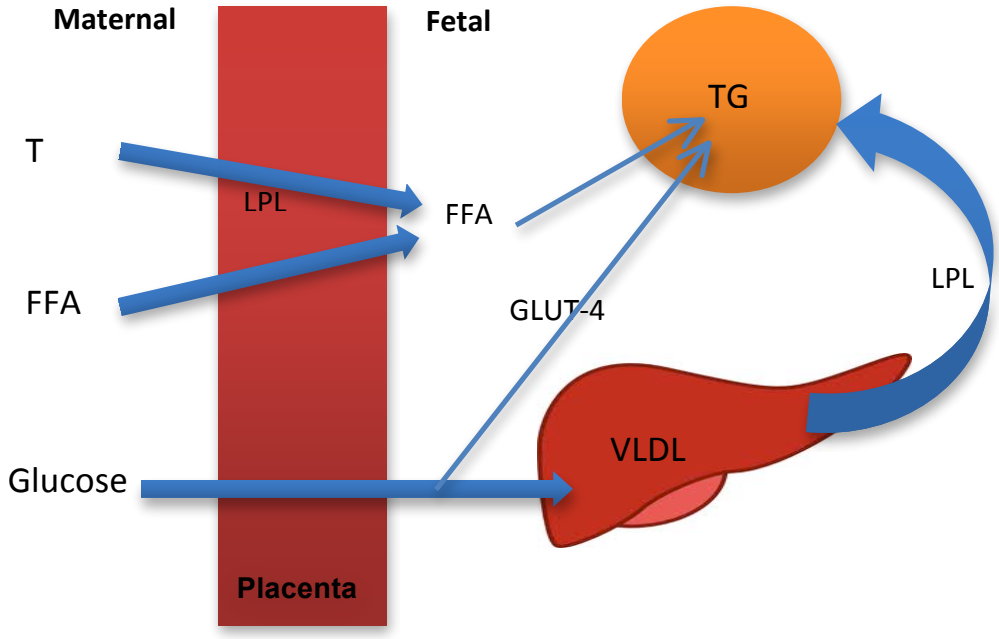


Figure 5 Energy substrates for lipogenesis in fetal adipocytes. Adapted from Catalano et al., 2011 (Catalano and Hauguel-De Mouzon 2011)

TG: triglycerides, FFA: free fatty acid, LPL: lipoprotein lipase, VLDL: very-low density lipoprotein

1.3.3.1 Lipotoxicity in obese women

Upper body and subcutaneous fat stores confer greater risk for cardiovascular disease than lower body depots and are associated with abnormal lipid metabolism (Wajchenberg 2000, Wajchenberg, Giannella-Neto et al. 2002, Schneider, Friedrich et al. 2010). Extending this concept further, evidence indicates that upper and lower body subcutaneous fat stores respond differently to the actions of insulin-mediated lipolysis with upper stores more resistant to insulin suppression of lipolysis thus generating approximately 60% of circulating NEFAs in comparison with 15-20% derived from lower body stores which are more sensitive to insulin (Jarvie, Hauguel-de-Mouzon et al. 2010).

Obese pregnant women preferentially store fat in visceral and upper locations compared to lean subjects suggesting cardiovascular protection for women who are

more “pear-shaped” (Ehrenberg, Huston-Presley et al. 2003) (Figure 6). As a consequence of increased dietary fat contribution and lipolysis, together with suboptimal storage, and impaired adipocyte maturation, concentrations of circulating NEFAs are significantly greater (Jarvie, Hauguel-de-Mouzon et al. 2010). This in turn promotes accumulation of ectopic fat particularly in the liver where cellular dysfunction occurs upon saturation with fatty acids.

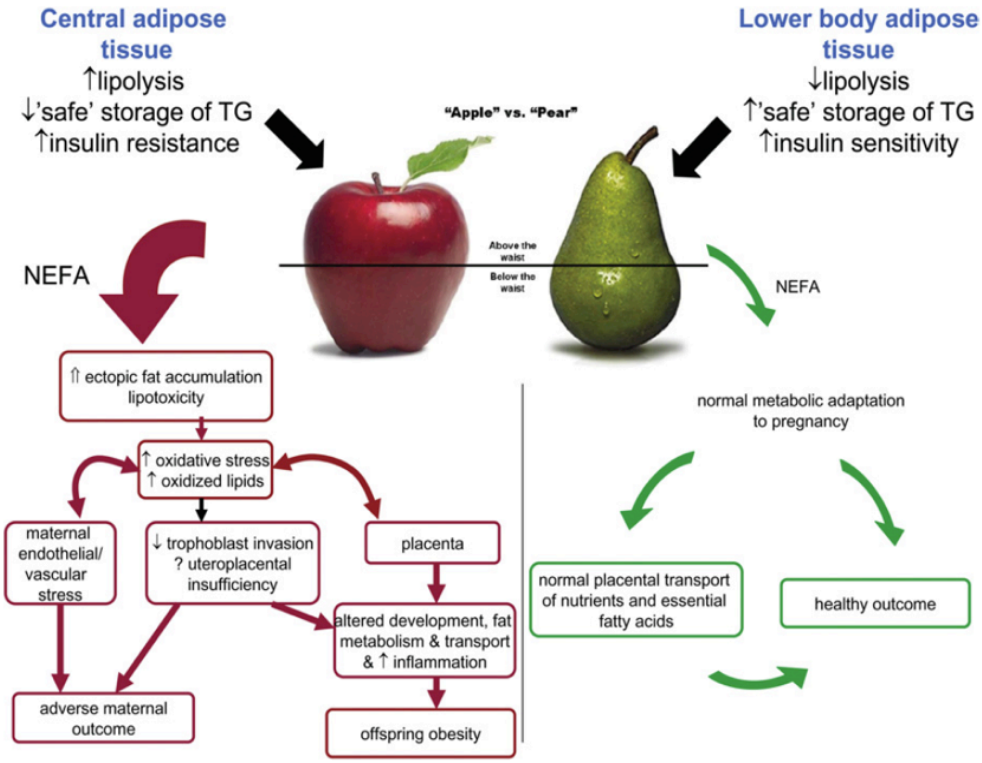


Figure 6 Fatty acid metabolism in pregnancy (Jarvie, Hauguel-de-Mouzon et al. 2010)

1.3.3.2 Mechanisms of insulin resistance

Prior to detailing individual biomarkers implicated in IR in obese pregnancy, an overview of the recent concepts and mechanisms of IR will be discussed.

Obesity is associated with a state of chronic inflammation, meditated by alterations in the concentration of circulating cytokines, reflecting abnormal changes to inflammatory and endocrine pathways and vascular dysfunction (Lee, Wu et al. 2013). Many of these biomarkers are implicated in the pathogenesis of T2DM and GDM although causality cannot be assumed because of residual confounding (Cao 2014).

Cellular mechanisms of insulin resistance

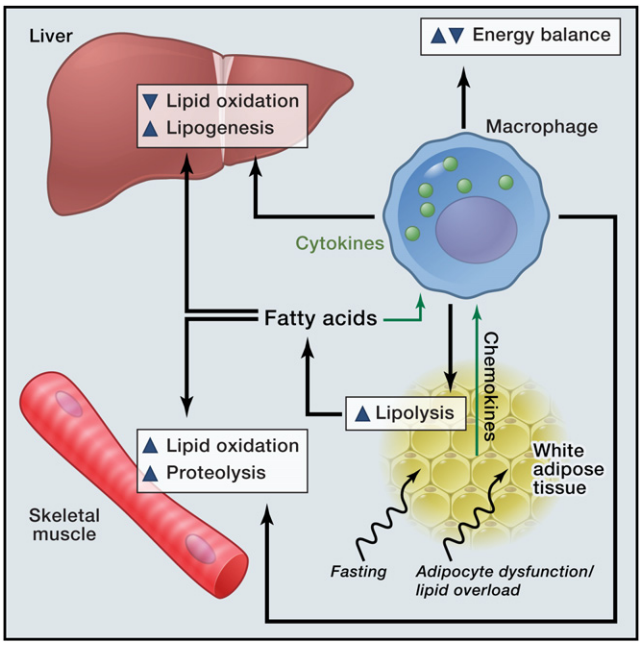


Figure 7 Summary of inflammation and changes to energy metabolism secondary to adipocyte dysfunction in obesity (Samuel and Shulman 2012)

The main sites involved in the normal cellular cycle of energy homeostasis are the liver, skeletal muscle and adipose tissue with additional signaling from the brain. Following insulin secretion, inhibition of hepatic gluconeogenesis occurs with the promotion of lipogenesis and glycogenesis to conserve dietary sources of energy. Glucose transport into skeletal muscle increases with similar accumulation of glycogen stores and inhibition of lipolysis in adipose tissue (Figure 7).

In obesity, the excess of dietary fuels coupled with reduced energy expenditure alters the cellular environment significantly, giving rise to various hypotheses on the complex aetiological pathways of IR. Lipotoxicity, inflammation, hypoxia and mitochondrial dysfunction with associated endoplasmic reticulum stress (ERS), also known as the unfolded protein response (UPR), have all been cited, with actions in the liver and skeletal muscle.

A complete understanding of the interrelationship of the multiple pathways involved in IR in humans is not yet known but extensive work using animal models, summarised in two detailed reviews discussed here, have provided greater insights into the cellular mechanisms involved (Muoio and Newgard 2008, Samuel and Shulman 2012).

Lipids

Ectopic lipid accumulation and high concentrations of circulating free fatty acids (or non-esterified fatty acids, [NEFA]) alter intracellular signaling. Following cellular entry, esterification of fatty acids by coenzyme A followed by coupling with a glycerol backbone leads to the formation of secondary messengers diacylglycerol (DAG) and ceramide which have been associated with IR in mouse models and humans. Ceramides also have a structural role in the cell membrane where they are found in high concentration. Diacylglycerols activate members of the protein kinase C family (PKC), which catalyze the phosphorylation of a wide variety of protein targets and are also involved in diverse signaling pathways.

Excess lipids in skeletal muscle are associated with activation of the novel PKC isoform PKCθ, giving rise to the potential link between increased lipid concentrations and altered cellular signaling in IR. Other novel isoforms including PKCε and PKCδ have been implicated in both muscle and hepatic IR via changes to insulin receptor substrate 1 (IRS-1). IRS-1 is a signaling adapter protein which has a key role in transmitting signals from extracellular insulin and insulin-like growth factor (IGF-1) to intracellular pathways e.g. the MAPK pathway (mitogen-activated protein kinases), which communicates signals to nuclear DNA following tyrosine phosphorylation of IRS-1.

Considering the central role of the liver in glucose homeostasis, hepatic steatosis as seen in non-alcoholic fatty liver disease (NAFLD), a condition characterized by insulin resistance and obesity, is a significant contributor to systemic IR. The direct relationship between hepatic IR and ectopic fat is illustrated in congenital lipodystrophy (CL), a rare autosomal recessive condition, where subjects have minimal subcutaneous fat stores and thus deposit fat ectopically; consequently, they develop hepatic steatosis and marked IR. Studies in lean subjects with CL have

shown that acute treatment with leptin to reduce appetite, lowers caloric intake and promotes a reversal in the histology and degree of IR (Petersen, Oral et al. 2002). In obese subjects with poorly controlled T2DM, a hypocaloric diet produced similar findings with an 85% reduction in hepatic fat, accompanied by improvements in hepatic insulin sensitivity and fasting glycaemia after 8 weeks on the diet (Lim, Hollingsworth et al. 2011). These results highlight the significant contributory role of the liver in systemic IR however do not confirm exclusivity in the process that is clearly a combination of altered cellular function within a number of tissues.

Unfolded protein response

The unfolded protein response (UPR) is initiated secondary to the accumulation of unfolded or misfolded proteins within the lumen of the cellular endoplasmic reticulum (ER). The aim of the UPR is to restore normal cellular function through activation of signaling pathways to produce chemical "chaperones" involved in protein folding. This process is mediated by three "arms" of the UPR, inositol requiring enzyme-1 (IRE1α), PKR-like ER kinase (PERK) and activating transcription factor-6 (ATF6), which reduce unfolding by three methods; increasing membrane synthesis and concentration of chaperones and preventing further protein translation. Chaperones that induce the UPR are known to impair insulin signaling whereas those that reduce the UPR improve it, suggesting an association with IR (Ozcan, Cao et al. 2004, Ozcan, Yilmaz et al. 2006).

Hypoxia

A growing body of evidence in obese and lean healthy subjects has confirmed strong associations of chronic and short-term systemic hypoxia with IR and progression to T2DM (Foster, Sanders et al. 2009, Siervo, Riley et al. 2014). Obstructive sleep apnoea (OSA), characterised by recurrent airway collapse, reduced sleep duration and intermittent sleep, affects 60-80% of obese subjects who are at significantly greater risk of multiple morbidities including T2DM, cardiovascular disease and various cancers. Potential mechanisms linking OSA with glucose intolerance in obesity include increased adipocytokine concentration and the generation of reactive oxygen species (ROS), perturbations in the hypothalamic-pituitary-adrenal (HPA) axis and activation of the autonomic system (Aurora and Punjabi 2013).

Furthermore, the failure of angiogenesis to meet the demands of hypertrophied and increased adipocyte mass in obesity is thought to promote hypoxia.

The role of hypoxia in systemic IR may be independent of hepatic and obesity related pathways. Rodent studies by Iiyori et al., examined the effects of acute hypoxia on measures of IR following hyperinsulinaemic euglycaemic clamp studies, in lean and otherwise healthy animals following a 9 hour exposure period (Iiyori, Alonso et al. 2007). Intermittent hypoxia was associated with acute insulin resistance and reduced skeletal muscle glucose utilisation however interestingly there was no change to hepatic basal glucose output in these non-obese animals, suggesting a potentially greater hepatic contribution to systemic IR with obesity.

Recent data from the Caudwell Xtreme Everest Research Group has shown that chronic (hypobaric) hypoxia has strong associations with progressive IR in healthy subjects in the context of active and significant weight loss (Siervo, Riley et al. 2014). A collaborative UK team ascended Mount Everest to the summit (8848m) over a total of 8 weeks. As the partial pressure of oxygen steadily declined (SpO_2 98% at sea level and 82% at 5300m), concentrations of fasting insulin and C-peptide increased by more than 200%, associated with a paralleled rise in the counter-regulatory hormones glucagon and adrenaline and mean weight loss of 7.3k±5.0kg ($p<0.001$). Despite these hormonal drives to mobilise energy stores and increase plasma glucose in response to the physiological stress, levels remained stable throughout indicating adequate pancreatic β-cell compensation. Markers of oxidative stress including glutathione, 4-hydroxy-2-neonenal (4-HNE) and isoprostanes were sequentially measured in addition to the inflammatory biomarkers Il-6, TNFα and macrophage inhibitory factor (MIF). Positive correlations were observed for HOMA-IR with both Il-6 ($r=0.74$, $p<0.001$) and 4-HNE ($r=0.42$, $p<0.001$) and an important association between oxidative stress (4-HNE) and inflammation (Il-6) was identified ($r=0.48$, $p<0.001$).

A review of the current evidence has shown that the presence of obesity and other co-morbidities such as OSA, make an important contribution to IR and influence the contributory role of the mediators involved.

Inflammation

Unlike the other mechanisms of IR discussed in this section which can be independent of obesity, the relationship of inflammation and T2DM, appears to be mediated by nutritional excess and increased adipose stores (Figure 8) (Hotamisligil 2006). Over the past decade, the significant role of inflammation in the development of chronic diseases has become accepted. The positive net effect of acute inflammation in tissue repair remains important, however the deleterious associations of chronic low-grade inflammation with cardiovascular disease, neurodegenerative conditions and multiple cancers have provided mechanistic insights and identified pathways for the development of possible therapeutic interventions (Couzin-Frankel 2010).

In the obese state, adipose tissue expresses a number of pro-inflammatory cytokines of differing primary origin, most likely as a consequence of immune cell infiltration in response to the expanding adipocyte (Table 6). The production of further chemotactic signals increases macrophage recruitment and potentiates the inflammatory response. Histological examination of fat tissue in obese mice and humans by Cinti et al., found that >90% of macrophage infiltration was localised to adipocyte cell debris, suggesting a possible clearance role although it is unclear whether apoptosis is a direct response to obesity or inflammation (Cinti, Mitchell et al. 2005). Tumour necrosis factor (TNFα) and interleukins IL-1, IL-1β and IL-6 have strong associations with T2DM (Donath and Shoelson 2011) and have been proposed as candidates for prediction of T2DM in combination with the acute phase protein CRP, synthesized by the liver in response to IL-6 stimulation (Wannamethee, Lowe et al. 2007, Wannamethee, Sattar et al. 2008, Lee, Adler et al. 2009). Interestingly a prospective study by Wannamethee et al., found that in 3,599 men aged 60-79 years with 108 incident cases of T2DM, the association of IL-6 with T2DM was independent of obesity and IR, in contrast to leptin (Wannamethee, Lowe et al. 2007).

Two intracellular signaling pathways, NF-κB and JNK appear to have an important role in the pathogenesis of T2DM in response to metabolic stress and inflammation via activation of the kinases IκB kinase-β (IKKβ) and JUN N-terminal kinase (JNK) (Donath and Shoelson 2011). IKKβ activates the transcription factor nuclear factor-

κB (NF- κB), which in turn promotes further inflammatory gene expression in the liver and adipose tissue. The increase in secretion of TNFα, IL-1β and IL-6, not only affects local tissues but more distant sites including skeletal muscle, the myocardium and vascular endothelium (Donath and Shoelson 2011).

A significant increase in JNK activity has been observed in adipose and hepatic tissues in obesity. Exposure to ER stress, FFA and cytokines, particularly TNFα, activates JNK and downstream transcription factors ELK1, ATF2 and JUN. The detailed mechanism of aberrant signaling in IR is not fully understood but JNK inhibits insulin action via serine phosphorylation of IRS-1 in a similar fashion to inhibition by PKC as detailed earlier (Hirosumi, Tuncman et al. 2002).

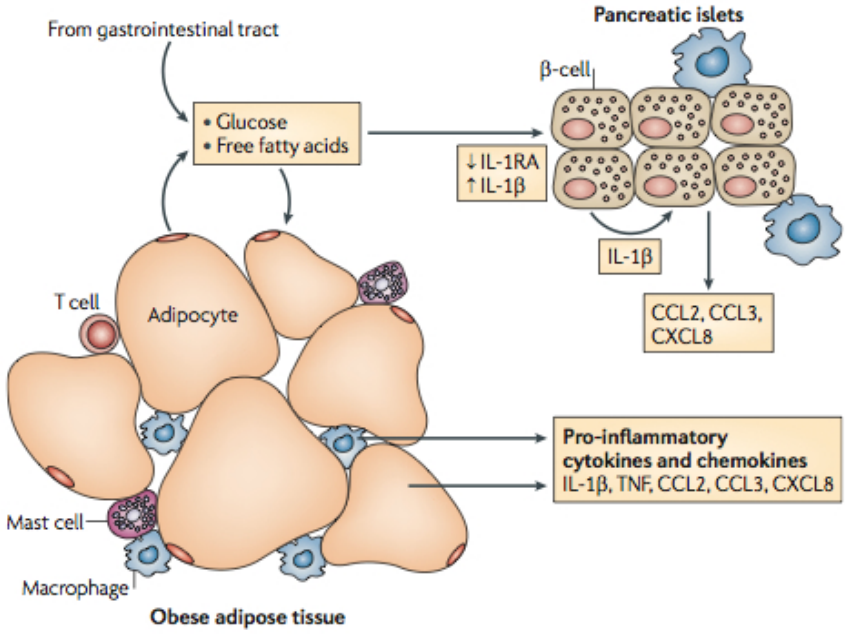


Figure 8 The cycle of inflammation in T2DM

In response to excess fuel-mediated stress, local production of cytokines and chemokines occurs in insulin-sensitive tissues: interleukin-1β (IL-1β), tumour necrosis factor (TNF), CC-chemokine ligand 2 (CCL2), CCL3 and CXC-chemokine ligand 8 (CXCL8). Beta-cell production of IL-1 reception antagonist (IL-1RA) is reduced, enhancing the immune response and inflammation both within the islets and systematically (Donath and Shoelson 2011).

Beta cell failure

Although obesity is associated with systemic IR and varying degrees of glucose tolerance, only a sub-set of those affected progress to T2DM, at a critical point when β-cell failure occurs.

Under normal conditions, glucose metabolism increases the ATP:ADP ratio, causing closure of ATP-sensitive K^+ channels and depolarisation of the plasma membrane. Voltage-gated Ca^{2+} channels within the cell wall are then activated, allowing an influx of Ca^{2+} required for vesicle release and exocytosis of insulin into the systemic circulation.

Other mechanisms of glucose stimulated insulin secretion, mediated by pyruvate generated from intracellular glucose metabolism, have recently been explored. Mitochondria of the β-cell express the enzymes pyruvate kinase (PC) and pyruvate dehydrogenase (PDH), which enable pyruvate entry, from glycolysis, into the tricarboxylic acid cycle (TCA cycle). This process of pyruvate cycling is part of an anapleurotic process where metabolic intermediates such as oxaloacetate and malate are generated within the TCA cycle prior to joining cytosolic pathways that ultimately return to pyruvate therefore promoting β-cell decompensation. These pyruvate cycling pathways are now known to be involved in glucose stimulated insulin secretion and identification of specific pathways is a focus of on-going research (Muoio and Newgard 2008).

The production and deposition of toxic amyloid fibrils produced by β-cells from islet amyloid polypeptide, is another potential mechanism of β-cell decompensation. Examination of islet cells from subjects with T2DM has confirmed the presence of amyloid fibrils within the plasma membrane which are associated with accelerated β–cell apoptosis, reduced first phase insulin response and low β–cell mass (Matveyenko and Butler 2006).

High-risk individuals have abnormal glucose tolerance for many years prior to a formal diagnosis of T2DM. From a review of the data, it is clear that the insidious onset of β-cell failure is multifactorial and most likely to be a consequence of metabolic overload in conjunction with oxidative stress and the UPR, increased cellular apoptosis, failures within the secretory mechanism of insulin granules and heightened genetic risk (Figure 9).

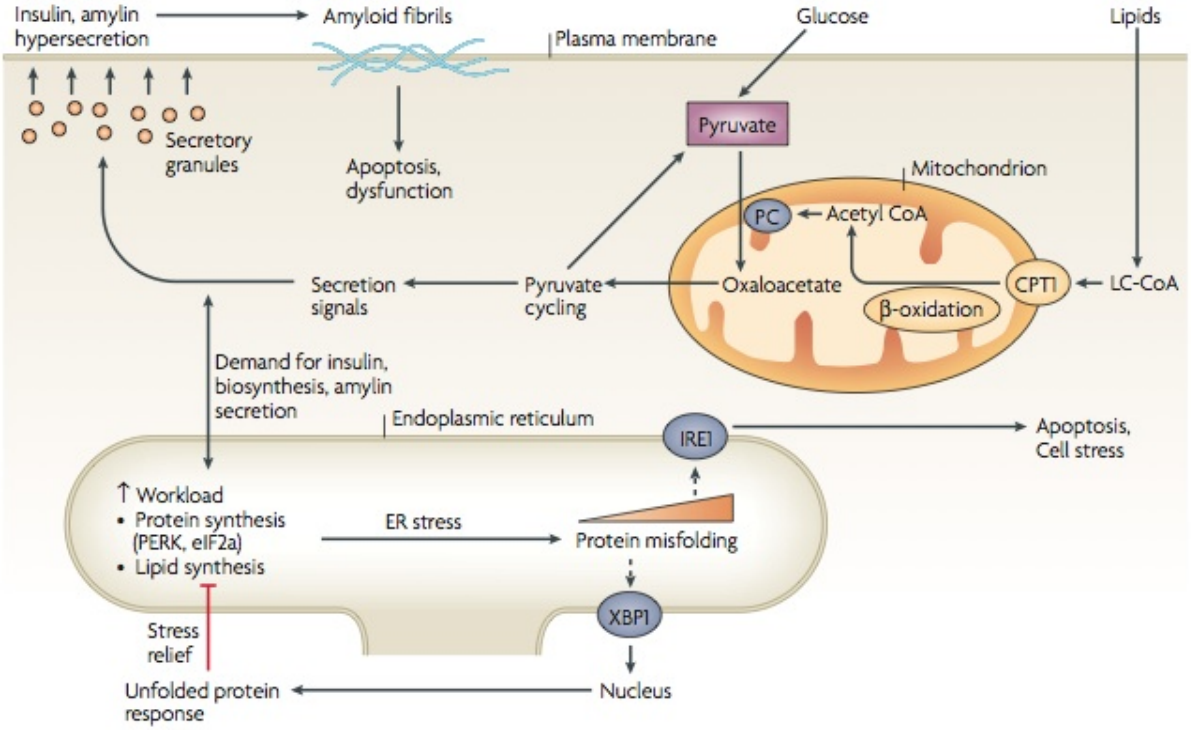


Figure 9 Mechanisms of β-cell failure in type 2 diabetes (Muoio and Newgard 2008)

Adipokines

Adipose tissue is considered to be an active endocrine organ, secreting a large number of peptide hormones and cytokines collectively known as adipokines. These include inflammatory mediators, angiogenic proteins and metabolic regulators, which possess paracrine and autocrine effects. Via complex signaling mechanisms that are not fully understood, adipokines are involved in modulation of blood pressure, inflammation, atherosclerosis, lipid and glucose metabolism. The production of adipokines is not exclusive to white adipose tissues (WAT) since secretion of certain adipokines has been identified in multiple tissues; in pregnancy, the role of the placenta as a source remains unclear (Sattar, Wannamethee et al. 2008).

Following expansion via increases in cellular number and hypertrophy, as seen in obesity, certain adipokines have been identified as being strongly associated with metabolic disease in adults, including pregnancy (Briana and Malamitsi-Puchner 2009).

Table 6 Summary of main cytokines implicated in the pathogenesis of metabolic dysfunction

Primary Source	Cytokine
Adipose tissue	Adiponectin (possible placental secretion)
	Leptin (placental secretion)
	Visfatin
	Resistin
	Retinol binding protein 4 (RBP4)
	Monocyte chemotactic protein-1 (MCP-1)
Macrophages and T-Cells	Interleukin-6 (IL-6)*
	Tumour necrosis factor (TNFα)*
Endothelium	Plasminogen activator inhibitor-1 (PAI-1)
	Tissue Plasminogen Activator (tPA)

*Also expressed by adipose tissue in obesity

Adiponectin

The plasma concentration of adiponectin, synthesized predominantly in adipocytes through expression of the ADIPOQ gene, is known to be inversely associated with improved glucose metabolism and appears to provide a good 'read-out' of whole body insulin sensitivity. In a recent meta-analysis low concentrations of adiponectin were associated with increasing BMI and found to be strongly predictive of type 2 diabetes (Li, Shin et al. 2009).

Low concentrations of adiponectin, as seen in obese women, have also been implicated with an increased risk of GDM. Differences have been observed not only at 24-28 weeks' coinciding with routine OGTT screening but as early as 11-13 weeks (Lacroix, Battista et al. 2013). More recently, significant associations between pre-pregnancy concentrations of adiponectin and subsequent GDM have been described in women with prior normal glucose tolerance (NGT) (Hedderson, Darbinian et al. 2013).

Hedderson et al. performed a nested case controlled study of women participating in the Kaiser Permanente Northern California Multiphasic Health Check Exam (1984-1996) with median time from blood test to pregnancy of 6.2 years. Women with GDM (n=256) had significantly lower concentrations of adiponectin (7.7μg/ml ±3.5 v 10.6μg/ml ±4.4, $p<0.001$). Linear regression analysis, combining the additive effects of low adiponectin (defined as <10.29μg/ml) and obesity (BMI≥25kg/m^2),

increased the odds of GDM 7 fold compared to lean controls with normal adiponectin concentrations (OR 6.7, 95%CI [3.6-12.5]) (Hedderson, Darbinian et al. 2013).

There is no compelling evidence thus far that pre-gravid concentrations of adiponectin change over the course of pregnancy for women with NGT or GDM, despite the normal accretion of additional adipose tissue and possible placental production of adipokines (Saben, Lindsey et al. 2014) however appropriate longitudinal data is extremely limited. In a prospective cohort of 445 women, of whom only 38 developed GDM, the change in adiponectin concentration from the 1st (6-13 weeks') to 2nd trimesters (24-28 weeks') was non-significant within or between the two groups (NGT -0.25±4.66 μg/ml, p=0.27; GDM 0.03±5.24 μg/ml, p=0.97 and between groups p=0.72) (Lacroix, Battista et al. 2013).

Other established adipokines

As a consequence of obesity, increased secretion of additional cytokines of different cellular origin, known to impair insulin signaling and thus compound IR, have been confirmed. These include adipose visfatin, leptin and resistin, IL-6 and tumor necrosis factor **(**TNFα) from macrophages and plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA) from the endothelium. In contrast to adiponectin, these all have a positive relationship with metabolic disease (Rasouli and Kern 2008, Sattar, Wannamethee et al. 2008).

Hepatic Biomarkers

As previously discussed (Lipotoxicity in obese women, 1.3.3.1, page 41), the storage capacity of adipose tissue is often exceeded in obesity. In turn promotion of ectopic fat deposition particularly in the liver occurs, affecting the secretion of hepatic derived biomarkers alanine transaminase (ALT), gamma-glutamyl transferase (GGT) and the acute phase protein, C-reactive protein (CRP), all associated with increased risk of incident T2DM (Fraser, Harris et al. 2009, Lee, Adler et al. 2009).

Maternal overweight and obesity

Several reviews have described the relationship between aberrant adipokine secretion with adverse metabolic consequences in pregnancy, notably GDM and PET but published data in specifically obese pregnancies are few (Briana and Malamitsi-Puchner 2009, Miehle, Stepan et al. 2012).

Ramsay et al. highlighted important changes in measures of glucose and lipid metabolism in the 3rd trimester (see 1.3.3, page 39) and were one of the earliest groups to confirm significant differences in the concentration of a selection of adipokines (Ramsay, Ferrell et al. 2002).

A continuous relationship between maternal glucose concentrations, C-peptide and BMI with selected inflammatory mediators at 24-28 weeks' gestation has now been confirmed by the authors of the HAPO study who examined a sub-set of 1481 women (mean BMI 28.4±4.8kg/m^2) (Lowe, Metzger et al. 2010). This unique and important study is the largest of its kind and shows an association between maternal glucose and inflammatory mediators in overweight women without diabetes. As FPG increased, mean adiponectin concentrations reduced, paralleled by rise in CRP and PAI-1; similar patterns were observed for 1 and 2 glucose values at OGTT for the same biomarkers, which remained significant following adjustment for BMI and C-peptide. Resistin was the only adipokine measured that did follow a continuous upwards trend across the same glucose categories used in the main study however the greatest mean concentration of resistin was found in the highest categories for BMI and C-peptide (The HAPO Study Cooperative Research Group 2008).

It has been proposed that the increase in inflammatory mediators in obese pregnant women may play a role in fetal growth regulation by modulation of placental nutrient transport in addition to maternal glucose homeostasis. In the HAPO cohort, adiponectin and CRP were inversely associated with birth weight, neonatal skin fold thickness and total body fat (estimated using anthropometry) and PAI-1 with sum of skinfolds only ($p<0.05$ for all) (Lowe, Metzger et al. 2010).

The inclusion of neonatal adiposity measures extending into early childhood is required in the design of future large studies, to explore this hypothesis further.

The Placenta

Rather than acting as a simple physiological conduit, the contributory role of the placenta in the overriding inflammatory state during an obese pregnancy is an area currently under investigation. Denison et al. measured the concentration and gene expression of a selection of pro-inflammatory cytokines such as IL-1b, IL-6, IL-8 and TNF-α in the placenta and plasma of 80 obese and non-obese women (Roberts, Riley et al. 2011). Of the cytokines measured, only plasma concentrations of IL-1b (3.03pg/ml±0.38 v 1.77pg/ml±0.15, p=0.001) were greater for obese women with a trend towards significance for TNF-α (1.8pg/ml±0.59 v 0.8pg/ml±0.16, p=0.06) whereas in placentas from the same women, expression of receptor m-RNA was significantly greater for IL-1b, IL-8, and the chemokine MCP-1 in obese women. The immune cell population was similar between the two groups and the only structural difference found, was an increase in placental blood vessel wall muscle thickness for obese women with equivalent macro and microscopic findings. It is unclear whether the vascular changes seen are a response or potential protective adaptation to the pro-inflammatory state in obesity.

Subsequent studies of smaller number (n=24) have reported alterations in the expression of genes related to lipid and cytokine activity, in keeping with placental inflammation and suggestive of a lipotoxic environment with increased oxidative stress (Saben, Lindsey et al. 2014).

1.4 Glucose homeostasis and mechanisms of insulin resistance in normal and obese pregnancies

Dynamic alterations in glucose homeostasis and insulin sensitivity occur from early through to late pregnancy to match the energy demands of the developing feto-placental unit. A 10-15% reduction in maternal fasting glucose is observed in the first trimester accompanied by changes in insulin secretion and increased peripheral sensitivity, particularly in skeletal muscle (Nelson, Matthews et al. 2010). Basal hepatic gluconeogenesis maintains normal glucose tolerance however with advancing gestation, a clear pattern of insulin resistance (IR) develops with a 50-70% decrease in insulin sensitivity and 200% increase in insulin secretion observed in normal pregnancies (Catalano, Tyzbir et al. 1992). Multiple systems and

interactions underlie the overall state of insulin resistance although exact mechanisms are not fully understood.

Normal adaptations to the hypothalamic-pituitary axis result in increased secretion of growth hormone and cortisol, paralleled by a rise in hepatic production of cortisol binding globulin (CBG) in response to placental oestrogens which serve to antagonise the effects of insulin (Karaca, Tanriverdi et al. 2010). Placental derived hormones particularly human placental lactogen (hPL) and human placental growth hormone (hPGH) have been shown to stimulate beta cell secretion of insulin in both animal and human islet studies (Brelje, Scharp et al. 1993) whilst maternal adipose tissue secretes multiple hormones and cytokines associated with IR and impaired insulin signalling including adiponectin, TNF-α, interleukin-6, leptin and resistin (Barbour, McCurdy et al. 2007). Increasing adiposity correlates with increased plasma concentrations of many of these proinflammatory adipokines, with the exception of adiponectin, however the influence of the distribution and accretion of fat depots on insulin resistance in pregnancy remains unknown (Kershaw and Flier 2004).

Characterisation of normal glucose profiles in pregnancy has been established using intermittent venous or capillary glucose measurements. The advent of continuous glucose sensor (CGMS) devices has enabled more precise determination of the pregnancy related changes to glucose homeostasis in both lean and obese subjects. Using CGMS, an early study by Yogev et al. examined 57 pregnant subjects during habitual living with no dietary restrictions over 72 hours (mean pregravid BMI 25.7±3.2 kg/m^2, mean gestational age 29.7±6.2 weeks'). Fasting blood glucose was 4.17mmol/l (SD 0.67) and mean blood glucose 6.11mmol/l (SD 0.89). Stratification of the group by BMI (obese n=15 and non obese n=42) revealed significant differences in glucose concentrations at defined time points. Obese women had a greater postprandial peak with increased time to achieve this and lower mean nocturnal glucose. No difference was observed for fasting and mean blood glucose concentrations (Yogev, Ben-Haroush et al. 2004). Harmon et al. furthered this work to evaluate patterns of glycaemia in early and late gestation in obese women without diabetes (early 15.7±2.0 and late 27.7±1.7 weeks gestation) but with significant differences observed between fasting as well as postprandial glucose values. Obese

women had significantly greater exposure to glucose from the 2nd trimester onwards and even with a small sample size (obese n=16 and lean n=22), they were able to demonstrate greater adiposity in infants born to obese mothers, measured by skin fold thickness which correlated strongly to late pregnancy concentrations of TG and free fatty acids (Harmon, Gerard et al. 2011). Irrespective of the diagnostic criteria used to diagnose GDM, obese women clearly have abnormal glucose tolerance in pregnancy compared to lean controls and remain at greatest risk of developing GDM.

1.4.1 Continuous glucose monitoring sensors

Continuous glucose sensors (CGMS) enable patients with diabetes on insulin therapy to improve self-management, avoid the undesirable effects of hypo and hyperglycaemia and enhance flexibility with daily activities and diet.

CGMS has multiple advantages over traditional self-monitoring of glucose (SMBG) in pregnancy. Precise timing of the postprandial glucose peak has not been established and there is no consensus on the optimal time to measure CBG to assess peak insulin response. Current accepted clinical practice varies from testing 60-120 minutes after a meal, on waking and prior to bed. Glucose fluctuations occur rapidly over 24 hours independent of mealtimes therefore periods of abnormal glucose concentration including nocturnal hypoglycaemia remain undetected by SMBG alone with potential clinical implications (Gardner, Wardle et al. 2011).

Further studies have demonstrated improved pregnancy outcomes with regards to lower birth weight, reduced risk of macrosomia and improved glycaemic control following changes in management as a result of CGMS intervention in women with T1DM (Murphy, Rayman et al. 2008). Using CGMS in research eliminates the errors observed by Mazze et al. of under and over reporting of glucose values, inclusion of phantom results and omission of data by subjects, identified when modified home glucometers with memory chips were used in parallel with traditional log-books (Mann, Beedie et al. 2013).

Given the utility of these benefits over SMBG in pregnancy, we focused on using this technology in this thesis to gather continuous glucose data that SMBG would

conceivably fail to capture. The technical aspect of CGMS will now be discussed in more depth.

Sensors provide data on the direction, magnitude, frequency and duration of glucose changes and using component software via a Bluetooth receiver, statistical reports are generated. Minimally invasive devices measure the glucose concentration of interstitial fluid (ISF) using subcutaneous electrochemical enzymatic coated sensors in place of capillary blood, which is used in conventional glucometers. Since glucose concentrations in the ISF are dependent on the rate of blood flow, change in blood glucose concentration and metabolic rate, a physiological mean lag time of 6.7 minutes occurs between the two measurements (Oliver, Toumazou et al. 2009).

The International Organization Standard (ISO) 15197 report states that for reference glucose values ≤4.2mmol/l the sensor should detect a concentration within 0.83mmol/l and for values ≥4.2mmol/l within ±20% (Louie, Markovic et al. 2013).

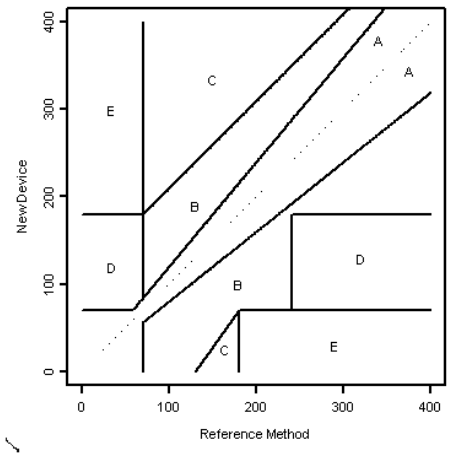


Figure 10 Clark error grid (mg/dl) (Oliver, Toumazou et al. 2009)

Manufacturers use Clark error grids (Figure 10), developed over twenty years ago, to assess the accuracy of their sensors. By plotting the sensor glucose value on the y-axis against reference glucose on the x-axis, a clinical risk score of five categories can be assigned to any glucose sensor error as follows: Zone A: clinically accurate, Zone B: benign, Zone C: overcorrect, Zone D: failure to detect and Zone E: erroneous (Oliver, Toumazou et al. 2009). The grid method assumes that target blood glucose is between 3.9-10.0mmol/l and values in zones A and B are deemed clinically acceptable.

Neither pregnancy with its associated hemodynamic changes nor sensor location (upper arm or abdomen) appear to affect the accuracy of sensor glucose concentrations measured, as demonstrated by Feldman et al. who reported 98% of readings in zones A or B using the FreeStyle® Navigator in subjects with T1DM (Abbott Diabetes, Alameda, California) (Feldman, Brazg et al. 2003). This sensor, which was selected for use in this thesis, can be worn for up to 5 days and requires five calibrations with capillary blood glucose (CBG) at 1, 2, 10, 24 and 72 hours post insertion.

1.5 Approaches to interventions in obese pregnancies

The majority of adverse complications of obese pregnancies are strongly associated with pre-pregnancy BMI (Nohr, Vaeth et al. 2008) therefore it would be reasonable to assume that pre-conception is the optimal time to intervene and reduce maternal weight however since a large proportion of pregnancies are unplanned, a significant proportion of women would potentially be missed. Pregnancy is perceived to be a time of high motivation for women not only to address and maximise their own health but that of their unborn child, creating a potential window of opportunity for long-standing lifestyle changes with a positive influence on future health.

Various early pregnancy intervention strategies including dietary (Wolff, Legarth et al. 2008, Thornton, Smarkola et al. 2009, Quinlivan, Lam et al. 2011) or lifestyle advice (Phelan, Phipps et al. 2011), physical activity (Barakat, Cordero et al. 2012, Oostdam, van Poppel et al. 2012) and combinations of the two (Guelinckx, Devlieger et al. 2010, Dodd, Turnbull et al. 2011, Vinter, Jensen et al. 2011) have been investigated to date. Comparison of studies is limited due to differences in primary outcome measures, BMI inclusion criteria and varying intensities and duration of interventions. Moreover few have tried to evaluate the effect of the interventions on behavioural change. Attempts to determine the most effective intervention type have been made with no clear outcome but following a meta-analysis by Gardner et al. of behavioural based interventions targeting diet and/or physical activity to reduce GWG, there is a suggestion that the most successful interventions are those started in early pregnancy and in women who do not smoke (Gardner, Wardle et al. 2011).

Furthermore, many studies to date have been considered to be of poor design and underpowered although several meta-analyses have sought to provide meaningful conclusions (Dodd, Grivell et al. 2010, Oteng-Ntim, Varma et al. 2012, Thangaratinam, Rogozińska et al. 2012) whilst large scale adequately powered RCTs with clinically relevant primary outcomes are currently in progress, using a variety of lifestyle (Dodd, Turnbull et al. 2011, Adamo, Ferraro et al. 2013, Poston, Briley et al. 2013) and pharmacological interventions (EMPOWaR ISRCTN 51279843 & MOP NCT01273584).

1.5.1 Dietary, lifestyle and physical activity interventions

Dietary and Lifestyle

Many studies are small but some of the larger ones are summarised here.

Dietary interventions have been developed to target various elements and patterns of food intake. Phelan et al. randomized 400 women from all BMI categories between 10-16 weeks' gestation to receive an intervention based upon reduction of high fat foods, increased exercise and self-monitoring of food intake until delivery (Phelan, Phipps et al. 2011). Education and reinforcement were made by an initial face to face visit with a health trainer who discussed appropriate weight gain within the 1990 IOM guidelines, advised 30 minutes of walking per day and set calorie goals (20kcal/kg). Subjects received weekly mailed material promoting healthy living and telephone feedback. Data from self-recorded food diaries was used in addition to demography taken in clinic visits at 30 weeks' and 6 months post partum. The intervention significantly reduced the number of healthy weight women who exceeded GWG recommendations compared to the control (40.2% v 52.1%, p=0.003) and whilst no such effect was observed in women who were overweight or obese, those in the intervention arm were twice as likely to return to pre-gravid weight or below at 6 months post partum irrespective of BMI (OR 2.1, 95% CI [1.3-3.5] p=0.005).

Similar reductions in GWG have been demonstrated following dietary intervention in two RCTs of comparable intensity for 132 and 360 obese women respectively (7.0kg v 13.8kg, p=0.04) (Quinlivan, Lam et al. 2011) (7.0kg v 8.6kg, p=0.01) (Vinter, Jensen et al. 2011). Results however are inconsistent, with no effect on

neonatal outcomes observed in the main (Thornton, Smarkola et al. 2009, Guelinckx, Devlieger et al. 2010, Quinlivan, Lam et al. 2011, Vinter, Jensen et al. 2011).

Luoto et al. were able to demonstrate a significant reduction in neonatal birth weight and incidence of LGA in women identified as high risk for GDM (BMI≥25kg/m^2, positive family history of T2DM, previous macrosomia and age ≥40yr) following a dietary and exercise intervention throughout pregnancy (8-12 to 37weeks') (Luoto, Kinnunen et al. 2011). Women of all BMI categories were included but conclusions were limited as a consequence of being underpowered. Despite this, these early studies have shown significant positive changes to the overall eating habits and lifestyle choices of obese pregnant women including diet composition and calorie intake, reduction of saturated fats, increases in polyunsaturated fats, improvements in fibre consumption and exercise undertaken (Wolff, Legarth et al. 2008, Guelinckx, Devlieger et al. 2010, Luoto, Kinnunen et al. 2011).

Exercise

Exercise is an established method of improving insulin sensitivity and overall glycaemic control in addition to reducing cardiovascular risk by influencing multiple clinical and biochemical variables associated with adverse events including HbA1c, SBP, anthropometry and triglycerides in subjects with T2DM (Chudyk and Petrella 2011). The acute effects of exercise can increase insulin mediated uptake by skeletal muscle by up to 40% for 16 hours, with long term effects from sustained activity thought to be mediated via increased GLUT 4 production (Duncan, Perri et al. 2003).

The progression to overt diabetes in high risk individuals including those who are obese with known glucose intolerance can be limited but the optimal mode, frequency, intensity and duration of exercise is yet to be identified (Eriksson and Lindgärde 1991, Tuomilehto, Lindstrom et al. 2001).

Advancing gestation and physical restriction coupled with maternal anxiety is often considered a barrier to exercise in pregnancy. Most exercise-only interventions centre around supervised exercise classes 2-3 times a week, adapted for pregnancy with a qualified trainer, with or without a home based element (Ong, Guelfi et al. 2009, Oostdam, van Poppel et al. 2009, Callaway, Colditz et al. 2010, Nascimento, Surita et al. 2011, Barakat, Cordero et al. 2012).

Barakat et al. instituted a moderate aerobic exercise programme of 35-45 minute sessions three times a week in lean pregnant women (mean BMI 22.7±2.8 and 23.3±2.9kg/m^2 for control and exercise groups) from early pregnancy (6-9 weeks' gestation) to term with an average of 85 sessions per women, assuming no preterm delivery. Group classes consisted of land and aquatic activities including light resistance training with full supervision and the support of an obstetrician (Barakat, Cordero et al. 2012). Maternal glucose screen at 24-28 weeks' revealed a significant reduction in venous glucose measured 1 hour after a 50g oral glucose load in the exercise group (5.77 v 7.10mmol/l [103.82±20.4 v 126.93±29.9mg/dl]) with no difference in GWG and incidence of GDM between the two groups. Importantly, the exercise protocol was deemed safe and acceptable to women with good adherence (Barakat, Stirling et al. 2008).

Conversely, the FitFor 2 programme found no significant differences in maternal FBG, fasting insulin, HbA1c, BMI or birth weight following a programme of similar intensity in women at high risk of GDM (BMI≥30 or >25kg/m^2 plus one other risk factor) (Oostdam, van Poppel et al. 2012). Lack of statistical power may have contributed to the results however compliance was poor with only 16.3% of women attending half of the total number of sessions (2/week) and as perhaps expected, compliance decreased with progression of pregnancy. No difference in the characteristics of compliant versus non-compliant women was noted from 15-24 weeks' but interestingly thereafter, compliant women were primarily Caucasian, nulliparous and educated to a higher level. Current evidence suggests that overweight and obese women may benefit the most from such exercise programmes in terms of greater reductions in GWG and measures of IR compared to lean women but translation into acceptable large scale RCTs is lacking (Ong, Guelfi et al. 2009, Nascimento, Surita et al. 2011).

Summary and safety of interventions

This current body of work has addressed potential concerns regarding the safety of such interventions. Thus far, no adverse effects of limiting gestational weight gain within recommended limits in obese pregnant women by dietary or exercise interventions have been reported (Barakat, Stirling et al. 2008, Thornton, Smarkola

et al. 2009, Vinter, Jensen et al. 2011, Stafne, Salvesen et al. 2012, Barakat, Pelaez et al. 2013).

However, caution should be heeded, because observational studies have highlighted deleterious consequences for those who gain less than 5kg (the lowest gain advised by the IOM for BMI≥30kg/m^2), including adverse effects on fetal growth. The largest review of data to address this issue is by Catalano et al., who performed a retrospective analysis of 1241 overweight and obese women who had previously participated in prospective studies (Catalano, Mele et al. 2014). The results suggest a greater risk of SGA in women who gained or lost <5kg during pregnancy (18/188 [9.6%] versus 51/1053 [4.9%] for those who gained >5kg) although the actual incidence of 9.6% is comparable to the background population rate of 10%. Infants had significantly less total and lean fat mass with smaller head circumference and length reflecting skeletal growth (Table 7). The risk of SGA was independent of maternal glucose status and the exclusion of all women with GDM from secondary analysis did not alter results.

Table 7 Comparison of neonatal measurements for restricted GWG (Catalano, Mele et al. 2014)

	Weight loss/gain <5kg (n=188)	**Weight loss/gain >5kg (n=1053)**	**P value**
Gestational age (weeks)	38.8 (1.4)	38.9 (1.4)	0.28
Gender			
Male	89 (47.3%)	540 (51.3%)	
Female	99 (52.7%)	540 (51.3%)	
Birth weight (g)	3258.4 (442.7)	3466.8 (491.5)	<0.0001
Length (cm)	49.3 (2.3)	50.0 (2.8)	0.001
Head circumference (cm)	34.2 (1.7)	34.5 (1.7)	0.02
Lean mass (g)	2855.1 (321.0)	2995.4 (346.9)	<0.0001
Fat mass (g)	403.4 (175.3)	471.4 (192.7)	<0.0001
Body fat (%)	12.0 (4.2)	13.2 (4.3)	0.0006
LGA	14 (7.5%)	139 (13.2%)	0.03
SGA	18 (9.6%)	51 (4.9%)	0.009

Data presented are mean (SD) or n (%)

It remains unclear whether limiting GWG further, causes undesired offspring changes as studies reporting a lack of potential harm have also been presented but

unlike the study by Catalano et al., detailed neonatal measures were not taken and results were presented for SGA and BW outcomes only (Blomberg 2011). The DALI study (ISRCTN70595832), a collaborative European RCT of a vitamin D and lifestyle intervention in different combinations for obese pregnant women is currently recruiting and may provide more insights into the effects of restricting GWG (target sample size 880). Women receiving dietary advice are advised to gain less than 5kg to achieve the primary outcome of GWG reduction. Prenatal ultrasound scanning will enable detailed intrauterine dimensions to be made and within 48 hours of birth, neonates will have full anthropometric assessment (Jelsma, van Poppel et al. 2013).

The effect of this weight reduction on clinically relevant outcomes is highly variable. In a recent meta-analysis, Oteng–Ntim et al. reported a significant reduction of 2.21kg in obese and overweight women with an associated lower incidence of GDM (OR 0.80, 95%CI [0.58-1.10]) but the strength of evidence was considered low. It is clear however that the optimal time to intervene for women at greatest risk for GDM is pre or early pregnancy (Gardner, Wardle et al. 2011, Tobias, Zhang et al. 2011) and evidence suggests that pre-pregnancy BMI may contribute as much to adverse outcomes as excessive GWG (Nohr, Vaeth et al. 2008).

1.5.2 Intervention studies aimed to improve glucose metabolism and reduce GDM incidence

Maternal obesity and GDM compound the risk of adverse pregnancy and long-term metabolic outcomes, therefore studies designed to not only reduce GWG but also reduce the insulin resistance of pregnancy in high-risk women are required. However, determining causality in positive studies to understand whether changes in IR are a consequence or independent of reduced GWG remains difficult. In the absence of any weight loss, improvements in peripheral insulin sensitivity and activity of lipoprotein lipase (LPL), an enzyme involved in the catabolism of triglyceride rich lipoproteins and strongly associated with IR, have been reported in sedentary adults following a six month walking programme suggesting that even modest exercise in pregnancy may improve glucose homeostasis (Duncan, Perri et al. 2003).

Considering the strong evidence to support lifestyle changes in reducing risk of T2DM (Tuomilehto, Lindstrom et al. 2001, Knowler, Barrett-Connor et al. 2002, Lindström, Louheranta et al. 2003), results of meta-analyses regarding interventions to reduce GDM are contradictory, with many unanswered questions (Tobias, Zhang et al. 2011, Yin, Li et al. 2013).

A meta-analysis by Tobias et al. included data for 34, 929 subjects, collated from five prospective cohorts, two retrospective case-controlled studies and two cross-sectional study designs with a total of 2855 cases of GDM. Strong inverse associations (25-55% reduced risk), between physical activity (PA) before or during early pregnancy and GDM were found, with the greatest magnitude of change observed for women engaging in the highest quantiles of activity before pregnancy (pooled OR 0.45 [95% CI 0.28 to 0.75], p=0.002) (Tobias, Zhang et al. 2011).

In contrast, a recent meta-analysis by Yin et al. identified only six RCTs of PA with a total of 1278 subjects (Yin, Li et al. 2013). Results were insufficient to support the use of PA to prevent GDM (risk ratio 0.91 (95% CI 0.57 to 1.44], p=0.68) however studies, inadequately powered to examine for GDM outcome, were included in the analysis (Callaway, Colditz et al. 2010).

Extending on earlier work, Barakat et al. completed the largest RCT of PA and GDM using the new international classification in 510 healthy Spanish women of normal BMI (24.1±4.1 kg/m^2 exercise group v 23.7±3.8kg/m^2 control, p=0.354) following the same protocol as detailed previously from 15 weeks' onwards (Barakat, Pelaez et al. 2013). Using both the WHO (2006) and IADPSG criteria to diagnose GDM, no reduction of GDM was seen between the two groups for either definition in keeping with findings from a large Norwegian study (n=702) that adopted a less intense intervention (Stafne, Salvesen et al. 2012). To ensure moderate exercise intensity was achieved, subjects used heart rate monitors and the Borg scale of perceived exertion however neither study measured the change in activity from enrolment to the endpoint, which would have provided information on potential behavioural change.

The normal pregnancy-associated rise in plasma insulin concentrations, can be attenuated by exercise, most likely via the same mechanisms observed in T2DM, at least in early gestation. A dietary intervention, designed to restrict GWG to 6-7kg, adhering to the official Danish dietary recommendations (fat 30E%, protein 15-20E% and carbohydrate 50-55E% [E%, % of total energy]) successfully limited GWG in obese pregnant women (6.6kg v 13.3kg, 95% CI [2.6-10.8], p=0.002) and reduced fasting plasma insulin concentrations from 15 weeks' onwards (Wolff, Legarth et al. 2008). At 27 weeks' a 20% reduction in plasma insulin was observed with a further 23% reduction at 36 weeks' (Figure 11). Corresponding group differences for insulin concentration were -16pmol^{-1} (95% CI [-32 to -1], p=0.04) and - 25pmol^{-1} (95% CI [-47 to -40], p=0.022) at 27 and 36 weeks' respectively. The intervention had no effect on FBG, 12 weeks' into the programme and although a significant reduction of 8% was observed at 36 weeks' (group difference -0.3g ml^{-1}, 95% CI [-0.6 to 0.0], p=0.03), translation of a relatively small glucose change into positive clinical outcomes in a larger sample size remains questionable since neonatal and maternal outcomes were the same for both groups including GDM. Two hour glucose values following OGTT were similar between the two groups throughout pregnancy.

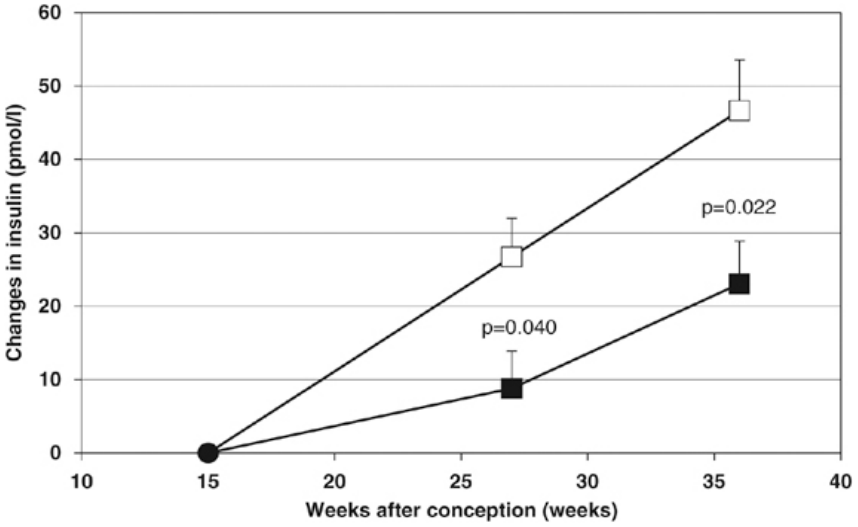


Figure 11 Changes in insulin concentration during pregnancy in obese women following a dietary intervention. Intervetion (■), Control (□) (Wolff, Legarth et al. 2008)

1.5.3 Targeted postprandial glucose interventions and the glycaemic index

To minimise the risk of excessive fetal growth secondary to maternal hyperglycaemia, therapeutic strategies aimed at achieving near-normal glucose concentrations without the undesired effects of hypoglycaemia are required.

Dietary advice remains the cornerstone of treatment for GDM, with increasing use of second line therapies particularly insulin and metformin for women who are unable to achieve adequate control with dietary changes alone (Dornhorst and Frost 2002, NICE 2015). Two seminal RCT's have unequivocally demonstrated that treatment of mild glucose intolerance in pregnancy improves neonatal and maternal outcomes (Crowther, Hiller et al. 2005, Landon, Spong et al. 2009). In the Australian Carbohydrate Intolerance Study in Pregnant Women (ACHOIS), women with mild glucose intolerance randomised to the intervention group received dietary advice and only commenced insulin when fasting and/or postprandial blood glucose targets were exceeded on more than two occasions. A significant reduction in serious perinatal complications was achieved in the intervention arm and although 20% of women in this group were commenced on insulin to optimize glucose control, it is important to highlight that the majority of women were successfully managed with dietary advice alone (Crowther, Hiller et al. 2005). Similarly in the Maternal-Fetal Medicine Unit (MFMU) study, treatment of mild GDM with diet and insulin (fasting BG<5.3mmol/l and 1-hr BG>10.0mmol/l, or 2-hr BG>8.6mmol/l, or 3-hr BG 7.8mmol/l following 100 gram OGTT) reported significant reductions in multiple secondary outcomes including mean birth weight, shoulder dystocia, birth weight >4kg, incidence of LGA and cesarean delivery (Landon, Spong et al. 2009).

The Low Glycaemic Index

Otto and Niklas first highlighted the different glycaemic response to various foods in 1980 with the subsequent concept of the glycaemic index (GI) food classification system developed by Jenkins in Toronto shortly thereafter (Jenkins, Wolever et al. 1981). Initial studies compared a 50g portion of carbohydrate calculated from published food tables to a standard of 50g glucose. Following consumption of the carbohydrate, serial venous blood samples were taken up to 2 hours and glucose values plotted to determine the area under the curve (AUC). Similar methods are

adopted today however use of capillary blood glucose (CBG) is more frequently used against a standard of white bread. The AUC for each food is expressed as a percentage of the mean AUC for the standard, from a sample of ten subjects and used to calculate the GI (Wolever, Jenkins et al. 1991). The GI predicts the ranking of the glycaemic response of foods in an individual; foods that are digested and absorbed slowly raise blood glucose concentrations gradually and are thus given low GI (LGI) values. The division of GI values into three simple categories has facilitated a useable system for individuals and generated an additional tool for the delivery of dietary advice for healthcare professionals; high GI ≥70, medium GI 56-69 and LGI <55.

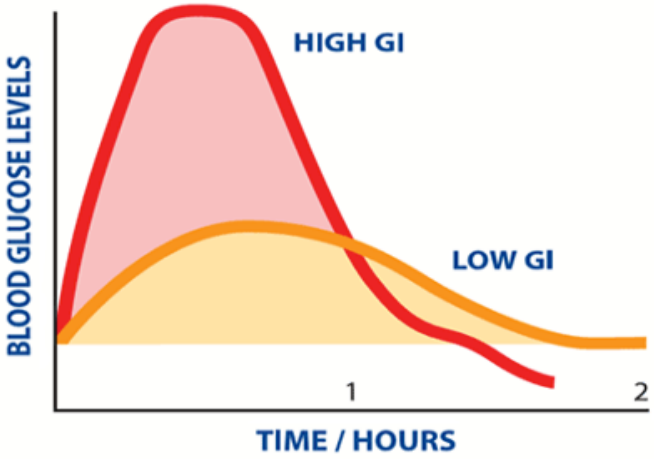


Figure 12 Schematic representation of the glycaemic response following consumption low and high GI food

Inherent and important limitations of GI classification exist when applying the principles to the wider population and when interpreting individual responses to foods of different categories. Aberrations in methodology are known to affect the glycaemic index including food portion size and preparation, choice of standard, timing of test, frequency of blood sampling and the formula used to determine the AUC (Wolever, Jenkins et al. 1991). Subject characteristics such as age, sex, ethnicity, degree of glucose intolerance and co-existing disease that may influence glucose absorption are also confounders but attempts to standardise these factors would serve to limit the variability in glucose response and maintain consistencies across food groups tested therefore minimising the effect on overall GI (Pi-Sunyer 2002).

Low GI and obesity

The evidence for the use of LGI diets in clinical scenarios has been evaluated in a series of systematic reviews from the Cochrane Library (Thomas, Elliott et al. 2007, Tieu, Crowther et al. 2008, Thomas and Elliott 2009, Han, Crowther et al. 2013).

The 2007 review for obesity (in non-pregnant subjects) included six eligible RCTs with a total of 202 subjects. The duration of LGI diet was wide, ranging from 5 weeks' to 6 months and with only 2 studies including a follow-up period, interpretation of results beyond the study duration is limited. Obese and overweight subjects who followed the LGI diet had significantly greater weight loss (weighted mean difference -1.1kg, 95% CI [-2.0 to -0.2]) and reductions in total cholesterol and LDL concentrations compared subjects on standard or high GI diets (WMD -0.22 mmol/l, 95% CI [-0.43 to -0.02] and WMD -0.24 mmol/l, 95% CI [-0.44 to -0.05] for standard and high GI respectively) (Thomas, Elliott et al. 2007). Subsequently, Liu et al. demonstrated a significant reduction in serum glucose and insulin AUC (42% and 29% respectively) when comparing diets of LGI/low carbohydrate to high GI/high carbohydrate in overweight and obese people (Liu, Most et al. 2012). The reduction in glycaemia and insulinaemia was sustained over the course of the 12 hour test day, following early morning test meal consumption, suggesting a prolonged effect of an LGI diet.

Low GI and diabetes

The Cochrane review of 2009 and an earlier meta-analysis both concluded that a modest, yet clinically useful, reduction in glycated hemoglobin (HbA1c) of 0.5% can be achieved with a LGI diet in the management diabetes, equivalent to the changes observed with single-agent pharmacotherapy (Brand-Miller, Hayne et al. 2003, Thomas and Elliott 2009). Similar limitations of small sample size and variation in duration of diet were reported and both papers included participants with T1 and T2DM, which differ significantly in their pathogenesis; the former mediated via autoimmune destruction of pancreatic islet cells and the latter via multiple altered pathways involving vascular, hepatic, inflammatory and adipose dysfunction, contributing to insulin resistance. The included studies did not detail changes to

weight or biomarkers associated with T2DM, which may have provided some mechanistic insight into how LGI carbohydrates influence glucose homeostasis.

Low GI and pregnancy

At present, data from two systematic reviews is inconclusive to support universal recommendation of LGI diets to prevent or treat GDM in pregnant women (Tieu, Crowther et al. 2008, Han, Crowther et al. 2013) however dietary GI is associated with maternal glucose metabolism and fetal outcomes for all women and not just those with GDM.

The Camden study was a prospective analysis of dietary GI calculated from three 24 hour recall questionnaires over the course of pregnancy, to explore associations between GI, glucose metabolism and fetal outcomes in 1082 women. A positive and significant relationship between GI, maternal HbA1c and plasma glucose 1 hour following 50g GCT was observed at 24-28 weeks' gestation. Using co-efficients from regression analysis, the authors calculated expected glucose values for the top and bottom quintiles of GI and found reductions for both in favour of an LGI diet: a difference of 2.2% and 4.2% for HbA1c and plasma glucose respectively was observed (Scholl, Chen et al. 2004). Similarly, GI was positively related to fetal growth. Following adjustment for GA only, a GI in the lowest quintile was associated with a 100g reduction in BW that increased to 116g when all possible confounders were included.

For women with established GDM, the adoption of an LGI diet following unsuccessful attempts to control glycaemia with generalised dietary advice can significantly reduce the progression to insulin therapy, without compromise to pregnancy outcomes (Moses, Barker et al. 2009).

Louie et al. conducted a systematic review of LGI diets in pregnancy for women of all BMI categories including three studies complicated by GDM (Louie, Brand-Miller et al. 2010). Of the eight studies included, half showed positive influence of a LGI diet on pregnancy outcomes, with a reduction in LGA reported in two but a moderate increase in the delivery of an SGA infant in one. A study by the same authors, included in the review, compared the effects of a LGI versus moderate-high GI (HGI) diet from 12 weeks gestation in a fairly low intensity study of 5 face-to

face consultations with no inclusion BMI restriction (n=70) (Moses, Luebcke et al. 2006). Both diets were in line with Australian guidelines for nutrition in pregnancy (dietary intake of fat 30% and carbohydrate 55%) and no specific requirements were made about total energy, fat or fibre intake. Women in the LGI arm, had infants with significantly lower birth weight, birth centile and ponderal index but rates of SGA for both arms were the same. The positive reductions in fetal size were independent of GWG and insulin resistance (HOMA-IR and fasting insulin), which were similar between the two groups at 36 weeks' and although women in the HGI were statistically heavier at enrollment, the actual difference in BMI was small (BMI: LGI 24.4±0.7 kg/m^2 and HGI 26.6±0.9kg/m^2, p=0.04).

In the main, LGI diets have been shown to be safe in pregnancy with no adverse maternal effects reported but issues regarding restricted fetal growth have been identified in the previously discussed Camden study. A small but increased risk of SGA for women with a dietary GI in the lowest quintile (GI <50) compared to those in the third quintile (GI 54-56) was observed with no change to the incidence of LGA with increasing GI. These concerns surrounding birth weight, identified by this prospective study, have not been reproduced in subsequent studies and will continue to be addressed by ongoing larger intervention trials.

1.5.3.1 Randomised controlled trials of LGI interventions in high risk pregnant women

The three largest RCTs aimed at lowering the GI of the diet, in conjunction with supporting a healthy lifestyle in high risk pregnant women have completed recruitment (Walsh, McGowan et al. 2012, Briley, Barr et al. 2014, Dodd, Turnbull et al. 2014) and two have published the outcomes (Walsh, McGowan et al. 2012, Dodd, Turnbull et al. 2014). Whilst the Pregnancy and Glycaemic Index Outcomes Study (PREGGIO) from Australia did not specifically recruit obese women and included those with NGT, the results are nonetheless relevant and merit discussion (Moses, Casey et al. 2014). The study evaluated the effect of LGI dietary advice compared to routine healthy eating with both arms receiving equivalent support and contact from the research team and dietician from 20 weeks' onwards. In contrast to

an earlier but smaller study from the same group (Moses, Luebcke et al. 2006) (see 1.5.3, page 66), no significant differences were observed for the primary outcomes of birth weight, birth centile or ponderal index. The failure to replicate the findings in the larger cohort with women of similar characteristics, is perhaps unsurprising since the control in PREGGIO was a healthy diet, re-enforced with dietary counselling, in contrast to the high GI diet, reflective of habitual diet, used in the earlier study.

ROLO (ISRCTN 54392969)

The ROLO study hypothesized that an LGI diet would reduce recurrence of macrosomia in subsequent pregnancies for women who had previously delivered an infant >4000g versus standard antenatal care with no dietary advice (n=781, mean BMI 26.8kg/m^2) (Walsh, Mahony et al. 2010). No difference in the primary outcome was observed but positive findings were noted for those following the LGI diet in relation to reduction of GWG and improvements in glucose tolerance. At 40 weeks' gestation, women following the LGI diet gained 1.5kg less weight than controls (mean difference -1.3, 95% CI [-2.4 to -0.2], p=0.01) and fewer exceeded the 2009 IOM guidelines for GWG (38% v 48% for LGI and control respectively). Both FPG and 1 hour glucose following 50g glucose challenge test were significantly lower in the LGI group although the incidence of GDM following 100g glucose load was no different (Walsh, McGowan et al. 2012).

LIMIT (ACTRN12607000161426)

LIMIT was the first adequately powered lifestyle intervention RCT for obese and overweight pregnant women with 2212 participants recruited to a combined programme of dietary and exercise advice, in conjunction with behavioral strategies. This was delivered over three visits with a research dietician and reinforced by 3 additional telephone consultations with trained assistants (Dodd, Turnbull et al. 2014). Dietary advice was not principally centered on LGI principles but incorporated food exchanges, increased fibre and fruit consumption and reduction of refined sugars to maintain a eucaloric diet and a global effect of lowering overall GI

(Dodd, Turnbull et al. 2011). The primary outcome of a reduction in LGA incidence (birth weight ≥90th centile for gestational age and sex) was not met against the control of routine antenatal care. Pregnancy outcomes including diagnosis of GDM and GWG were the same apart from a small reduction in risk for the secondary outcome of macrosomia in the intervention group (15% v 19%, RR 0.83, 95% CI [0.68-0.99], p=0.04).

UPBEAT (ISRCTN 89971375)

The (UK Pregnancies Better Eating and Activity Trial) UPBEAT, which has recently completed recruitment and will soon report on clinical outcomes, is multi-center RCT of a complex dietary and physical activity intervention aimed at improving glucose homeostasis in obese pregnant women (n=1555). Following randomisation at 15^{+0}-17^{+6} weeks' gestation to intervention or standard antenatal care, the programme is delivered over eight weekly group sessions with a health trainer until OGTT at 27^{+0} – 28^{+6} weeks' gestation. The objective of the dietary recommendation is to reduce the GI and glycaemic load (GL= GI x carbohydrates (g)/100) using food swaps and to exchange saturated fatty acids (SFA) for monounsaturated (MUFA) and polyunsaturated fats (PUFA). Women maintain contact with the research team with three further structured visits until delivery where dietary data, blood samples and anthropometry are obtained. Within 72 hours of birth, a detailed anthropometric assessment of the baby is made which is repeated at the 6 month postnatal review.

The study endpoints include maternal and neonatal primary outcomes of GDM diagnosed by 75g OGTT using the recommendations of the IADPSG (IADPSG 2010) and LGA delivery defined as adjusted birth weight >90th centile for gestational age using customised centiles, where adjustment is made for maternal height, weight, ethnicity, parity, and infant sex (Gardosi, Figueras et al. 2011).

Extended details of the intervention and protocol will be discussed in methods (Chapter 3, page 78) and are available on the trial web site (http://www.medscinet.net/upbeat/about.aspx).

A pilot trial was undertaken in 183 women in four urban UK hospitals, to evaluate changes in dietary and physical activity behaviours in response to the intervention, to trial all aspects of the protocol and to undertake process evaluation (Poston, Briley et al. 2013). Baseline habitual diets for all women were similar with no differences in the following; total energy intake, GI, GL and macronutrient composition.

Table 8 Dietary outcomes for the UPBEAT pilot study at 28 weeks' gestation

	Control (n=69)	**Intervention (n=71)**	**Difference (95% CI)**	**P Value**
Energy intake (MJ/d)	7.71 (2.30)	6.75 (2.57)	-0.94 (-1.72 to -0.18)	0.016
Dietary GI (%)	60 (26)	53 (13)	-7 (-15 to 0)	0.054
Dietary GL (g/d)	146 (55)	111 (39)	-33 (-47 to -20)	<0.001
GL (%E)	31.3 (13.3)	26.6 (8.0)	-4.8 (-8.5 to -1.0)	0.013
Carbohydrate (%E)	48.2 (8.0)	50.0 (8.2)	1.7 (-1.0 to 4.4)	0.207
Protein (%E)	15.5 (3.2)	17.1 (4.9)	1.5 (0.1 to 2.8)	0.034
Protein (g)	70.6 (24.0)	66.5 (23.5)	-4.8 (-12.3 to 2.6)	0.204
Total fat (g)	35.9 (7.7)	32.5 (7.4)	-3.2 (-5.6 to -0.8)	0.010
SFA (%E)	12.9 (3.9)	11.1 (3.8)	-1.6 (-2.8 to -0.3)	0.015
MUFA (%E)	11.6 (4.0)	10.4 (3.2)	-1.0 (-2.2 to 0.2)	0.088
PUFA (%E)	5.9 (2.8)	6.0 (2.7)	0.13 (-0.8 to 1.1)	0.774
P:S ratio	0.51 (0.35)	0.64 (0.52)	0.13 (-0.01 to 0.28)	0.075
NSP (g)	10.5 (4.2)	12.0 (6.0)	1.77 (0.08 to 3.47)	0.040

Abbreviations: GI glycaemic index, GL glycaemic load, MUFA monounsaturated fatty acid, NSP non-starch polysaccharide, P:S ratio polyunsaturated fatty acid: saturated fatty acid ratio, PUFA polyunsaturated fatty acid, SFA saturated fatty acids, %E percentage energy

Results are given as mean (SD) for variables at 28 weeks gestation. Comparisons are adjusted for baseline levels throughout.

At 28 weeks gestation, there was a significant reduction in total energy intake, GL, total fat and SFA with an increase in fibre consumption (non-starch polysaccharides, NSP) for the intervention group (Table 8). Groups entered the study with a medium

GI diet (58) but after 8 weeks of adherence to the programme, a borderline significant 7-point reduction in the GI was achieved, with the intervention group diet reclassified as LGI (difference -7 [95% CI -15 to 0], p=0.054). On completion of the study, analysis will determine if these positive dietary changes are maintained throughout pregnancy and beyond and whether they translate into favourable clinical outcomes.

In summary, for all the pregnancy interventions discussed, the additional commitment over and above the time required for routine antenatal care is a severe limitation. Poor compliance and high dropout rates are greatest in women from ethnic minorities and those who have not received higher education (Callaway, Colditz et al. 2010, Vinter, Jensen et al. 2011, Oostdam, van Poppel et al. 2012). Authors have attempted to facilitate participation by various means including local delivery, provision of transport to research centres and regular phone, postal and email contact but greater understanding of barriers particularly to increased PA is needed.

Adequately powered RCTs are required to determine if sole or combination interventions are successful in improving maternal, obstetric and offspring outcomes and whether they have real translational potential as a therapeutic option for obese pregnant women. Pragmatic study designs that encourage and support pregnant women, which can adapt to meet the cultural requirements of the local population, are essential.

1.5.4 Prediction of GDM

Not all obese women develop GDM, however this heterogeneity poses a burden on limited resources with all women with a BMI$\geqq$30kg/m^2 currently managed as if at risk, often resulting in sub-optimal management. Accurate and early identification of pregnant obese women who will subsequently develop GDM women would enable early risk stratification, more appropriate use of health care resources and targeting of potential intervention strategies.

Currently, the UK National Institute for Health and Care Excellence (NICE) recommend selected rather than universal GDM screening, according to risk factors, which include obesity. Women who have previously delivered a macrosomic infant, have had previous GDM, or who have a first degree relative with diabetes and high-risk ethnicity are also screened. A systematic review of screening for GDM undertaken for a health technology assessment (HTA) reported low sensitivities (50–69%) and specificities (58–68%; eight studies) when traditional methods of risk factor screening were used (Scott, Loveman et al. 2002).

In this thesis, a preliminary investigation was undertaken to determine whether the addition of a panel biomarkers to routine clinical measurements further improves prediction of GDM. For this purpose we studied 117 women participating in a pilot trial for the UPBEAT study and sixteen biomarkers frequently implicated in the pathogenesis and prediction of GDM and/or type 2 diabetes. These reflect inflammatory pathways, markers of adipose tissue function, hepatic fat accumulation and vascular dysfunction (Sattar, Wannamethee et al. 2008, Savvidou, Nelson et al. 2010, Ferreira, Rezende et al. 2011). Prediction models were then developed incorporating clinical and biomarker data.

2 AIMS

2.1 UPBEAT

Hypothesis

The changes in fat distribution in obese pregnant women who develop GDM differ from those with normal glucose tolerance in pregnancy, with a preference for central and upper body accumulation and subsequent alterations in the secretion of adipokines. Using this information in conjunction with differences in clinical measures, more accurate prediction of GDM in obese women, than current strategies yield, can be made.

Specific aims:

In the UPBEAT pilot study:

1. Examine longitudinal changes in maternal biochemistry and adiposity.
2. Assess any effect of the intervention on maternal biochemistry, including non esterified fatty acids (NEFA) and adiposity.
3. Develop a prediction model to identify those at greatest risk of GDM, which shall be repeated on completion of the whole study.

2.2 Improving glycaemic profiles in obese pregnant women (IGPOP)

Hypothesis

The consumption of a slow digesting, low glycaemic index (SD-LGI) nutritional supplement in obese pregnant women will attenuate the postprandial glycaemic response compared to a eucaloric supplement of equal macronutrient composition with a corresponding reduction in plasma concentrations of insulin and C-peptide.

Specific Aims

1. To obtain data on glucose, insulin and C-peptide response in obese pregnant women following administration of a nutritional supplement compared to control supplement.
2. To examine the immediate and prolonged effects of consumption of a SD-LGI supplement on blood glucose concentration and further investigate the glucose lowering effect of the supplement when consumed as part of a mixed meal or in isolation as a snack.
3. To determine the normal glycaemic profile of obese pregnant women following habitual diet using continuous glucose monitoring.
4. To inform the design of a large scale RCT to demonstrate the benefits of the nutritional supplement on maternal and infant outcomes.

3 METHODS: UK PREGNANCIES BETTER EATING AND ACTIVITY TRIAL (UPBEAT)

The pilot study for UPBEAT was performed at 4 urban centres in the UK: Guy's and St.Thomas' NHS Foundation Trust (London), King's College Hospital Foundation Trust (London), The Southern General and Princess Royal Maternity Hospitals (Glasgow) and The Royal Victoria Infirmary (Newcastle), between March 2010 and April 2011.

Ethical Approval: NHS Research Ethics Committee approval was obtained in all contributing centres (UK IRAS integrated research application system; reference 09/H0802/5).

3.1 Recruitment

Eligible subjects were identified using maternal demographics used for outpatient clinic lists and were approached by a research assistant at the first antenatal ultrasound scanning appointment. Verbal and printed information was provided and followed up with a call from a research midwife (RMW) after a minimum of 24 hours. For those who were willing to participate, the 1st study visit was scheduled a week later between 15^{+0}-17^{+6} weeks' gestation and for those who declined, consent was sought to record basic demography including BMI. Active screening for undiagnosed T2DM in those with previous GDM with HbA1c or FBG was not performed. If available, postnatal data on diabetes status was used.

Inclusion criteria: Obese women, BMI≥30kg/m^2, with a singleton pregnancy between 15^{+0}-17^{+6} weeks' gestation and no history of GDM or glucose intolerance in the index pregnancy.

Exclusion criteria: pre-existing diabetes, metformin therapy, coeliac disease, pre-existing renal disease or essential hypertension, systemic lupus erythematosus (SLE), antiphospholipid syndrome, sickle cell disease or thalassemia and current psychosis.

A flowchart summarizing the study protocol and principle research visits, together with a brief description of data obtained at each visit in the UPBEAT pilot study is given below.

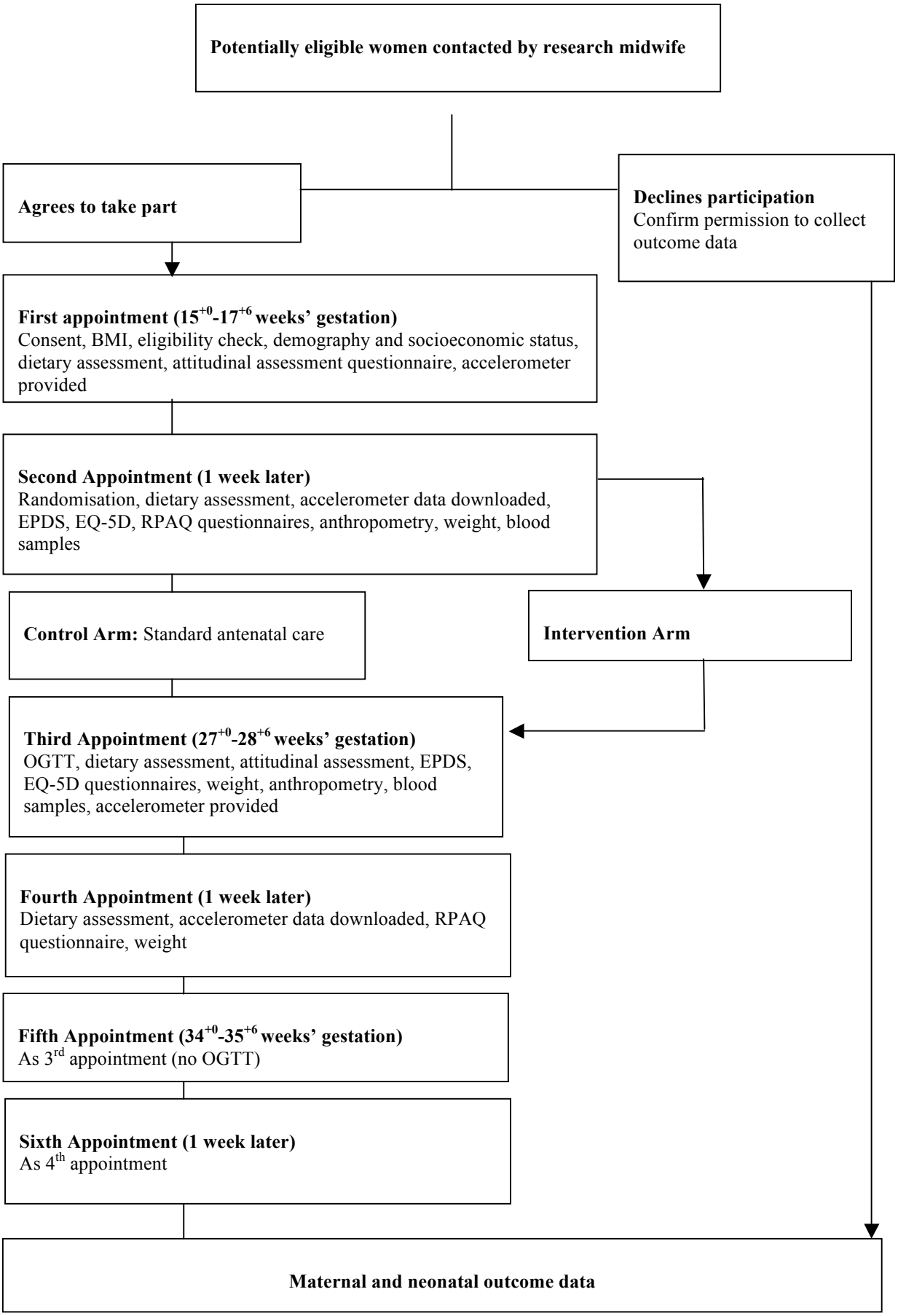
3.2 Protocol

Recruitment (15^{+0}-17^{+6} weeks' gestation)

Following informed consent, information was obtained on demography, maternal and family history and current pregnancy health. Randomisation to the intervention or control arms, which consists of standard antenatal care, occurred at the second appointment, approximately one week later (7-10 days) between 16^{+0} and 18^{+6} weeks' gestation by a secure internet based data management system (MedSciNet™). The randomisation schedule was minimised according to ethnicity, parity (0 vs >= 1), age and BMI (30-34.9kg/m^2 vs 35-39.9kg/m^2 and >40kg/m^2). For all centres, study visits and data collection took place in a clinical research facility or the antenatal outpatients department with a dedicated RMW.

Pre-pregnancy BMI was confirmed from patient hand-held antenatal notes documented at the first routine antennal visit with a midwife at approximately 8-12 weeks' gestation and measured at the first study visit to ensure eligibility. Height was measured using the free standing Leicester Measure® stadiometer and weight with the Marsden® digital scales in light clothing, without footwear. If BMI was <30kg/m^2 at this visit, subjects were excluded.

Blood pressure was recorded using the Microlife® BP3BT0-A automated blood pressure monitor which is validated for use in pregnancy and following appropriate training, maternal skinfold thickness (triceps, biceps, subscapular and supra-iliac) were measured in triplicate with Harpenden skinfold calipers (validated for values ≤80mm) (Holtain Ltd, Wales, UK) in addition to the following circumferences: waist, mid arm, thigh and hip. Total sum of skinfolds was calculated at four sites (triceps, biceps, suprailiac and subscapular).


Potentially eligible women contacted by research midwife
Agrees to take part
Declines participation
Confirm permission to collect outcome data
First appointment (15+0-17+6 weeks' gestation)
Consent, BMI, eligibility check, demography and socioeconomic status, dietary assessment, attitudinal assessment questionnaire, accelerometer provided
Second Appointment (1 week later)
Randomisation, dietary assessment, accelerometer data downloaded, EPDS, EQ-5D, RPAQ questionnaires, anthropometry, weight, blood samples
Control Arm: Standard antenatal care
Intervention Arm
Third Appointment (27+0-28+6 weeks' gestation)
OGTT, dietary assessment, attitudinal assessment, EPDS, EQ-5D questionnaires, weight, anthropometry, blood samples, accelerometer provided
Fourth Appointment (1 week later)
Dietary assessment, accelerometer data downloaded, RPAQ questionnaire, weight
Fifth Appointment (34+0-35+6 weeks' gestation)
As 3rd appointment (no OGTT)
Sixth Appointment (1 week later)
As 4th appointment
Maternal and neonatal outcome data

Blood and urine samples were obtained from 117 women in the three centres that had facilities for sample handling and storage. Serum and plasma was stored at -80°C for future analysis. Details of sample collection and processing are discussed in 3.7, page 84.

A series of questionnaires were completed to obtain data on behavioral and psychological well being; the EuroQuol Quality of life (EQ-5D), the Edinburgh Postnatal Depression Scale (EPDS), and the International Physical Activity Questionnaire (IPAQ). Detailed dietary data was collected using the triple-pass 24 hour recall method and the validated food frequency questionnaire (FFQ) at the start and end of the appointed week. Calculation of GI and macro/micronutrient composition was made using WISP Tinuviel software®. Physical activity (PA) was measured in all participants by accelerometry over two separate weeks prior to randomisation and post intervention at 27^{+0}-28^{+6} weeks'.

Post Intervention (27^{+0}-28^{+6} weeks' gestation)

At 28 weeks' gestation, following the 8 week intervention programme, all subjects underwent a 75g oral glucose tolerance test (OGTT) with GDM diagnosed according to the International Association of the Diabetes Pregnancy Study Groups (IADPSG) criteria (fasting blood glucose ≥5.1mmol/l or 1 hour glucose ≥10.0mmol/l or 2 hour glucose ≥8.5mmol/l)(IADPSG 2010). Women with GDM were referred to routine antenatal NHS care. At Guy's and St.Thomas' NHS Foundation trust, women with IADPSG GDM were only referred to routine NHS care if they met the local trust criteria for GDM. For those that did not meet these glucose thresholds, ongoing clinical care was provided by myself in a separate clinic with referral access to the main diabetes antenatal service if required.

Late pregnancy study visits

A further three structured visits were scheduled for the duration of the pregnancy at 29^{+0}-30^{+6}, 34^{+0}-35^{+6} and 36^{+0}-37^{+6} weeks' gestation for the purposes of assessing diet and physical activity (PA) over the period of one week. Blood samples were collected and maternal anthropometry measured at visit five only yielding three-time points for biochemical and anthropometric data in total:

1. Visit 2 (V2): randomisation [15^{+0}-17^{+6} weeks']
2. Visit 3 (V3): post intervention and OGTT [27^{+0}-28^{+6} weeks']
3. Visit 5 (V5): late pregnancy [34^{+0}-35^{+6} weeks']

Maternal and neonatal outcome data was recorded at delivery and at the 6 months postnatal review.

It should be noted that in keeping with pragmatic study design of UPBEAT, women only attended in the fasted state for the OGTT post intervention at visit 3 (27^{+0}-28^{+6} weeks'). Therefore all biochemistry analyses are measured on a random sample with only those at visit 3 fasted.

Primary and secondary outcomes

In this thesis the primary outcome was concentration of a panel of biomarkers measured at 28 weeks' gestation following the 8-week intervention (see 3.7.3.1, page 85 for table of 16 biomarkers).

Secondary outcome was the development of a prediction model for GDM.

3.3 Data collection

All data was imputed directly into a secure, password protected online database system developed and maintained by Medscinet® (http://www.medscinet.net/upbeat/about.aspx). Information for each centre was available for authorised persons to review and the clinical trial manager performed quality control on a regular basis.

On completion of the pilot, Microsoft® excel spread sheets of blood sample locations for each centre were generated using the online data export facility to minimize transcribing error and used to coordinate sample collection for analysis. All raw data used in the main analysis was exported in a similar way.

3.4 Intervention

The intervention was delivered in group sessions by health trainers over an 8 week period from 19 to 27^{+0}-28^{+6} weeks' gestation when OGTT was performed. For

subjects unable to attend all group sessions, contact was made via phone or email. Health trainers received appropriate training from the study dietician and Weight Concern, a registered charity (Number 1059686), with ongoing peer support provided throughout, in the form of educational away days for all research staff.

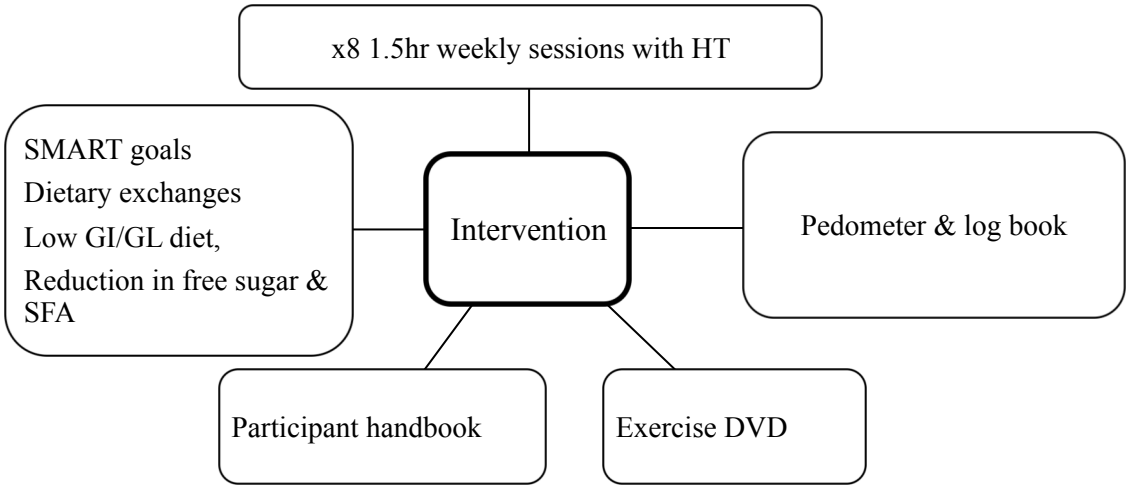


Figure 13 Component parts of the intervention adopted in the UPBEAT pilot study

Using the SMART model of behavioural change (specific, measurable, achievable, relevant and time specific) with positive feedback rather than setting predefined goals, women were encouraged to change their eating and PA behaviours with the aid of a log book (Doran 1981).

At the initial one-to-one visit, women were given the following; pedometer, log book for weekly SMART goals, DVD of pregnancy specific exercises and a participant handbook detailing the content and theory behind the health trainer (HT) sessions. At each group session, different elements of dietary and PA interventions were covered with a review of the previous week's SMART goals and new ones set for the future. Personal barriers to behavioural change were discussed amongst the group with potential solutions as to how these may be overcome generated.

Dietary advice

Specified dietary targets included a decrease in overall GI, glycaemic load (GL) and consumption of SFAs. A reduction in total energy intake was not a specific aim but this was an anticipated outcome of the dietary changes. Women were encouraged to exchange high GI for LGI foods, switch from drinks containing sugar to those

flavoured with LGI alternatives and replace saturated fats with monounsaturated and polyunsaturated fats. Dietary changes were discussed in the HT facilitated group sessions, supported by the use of visual aids and the participant handbook.

Physical activity

Women were advised to increase their PA by gradually increasing the time spent walking at a moderate level, using the pedometer as a tool to record and titrate up the number of steps taken.

3.5 Control

Women in the control arm followed standard antenatal care within each centre. They attended study visits at the 4 defined time points following randomisation for anthropometry, blood sampling and completion of questionnaires. Those diagnosed with GDM were seen by their hospital diabetes team according to local protocol.

3.6 Power Calculation

A power calculation to meet the specific aims of this thesis, that is to assess to effect of the intervention on the panel of biomarkers at 28 weeks' gestation and subsequently develop a prediction model for GDM, was not performed. Limitations surrounding this are raised in the discussion.

The total sample size (n=183) was obtained from the already completed UPBEAT pilot study, which was powered for a change in dietary and physical activity behaviours at 28 weeks' within a predefined time limit.

3.7 Blood Samples

3.7.1 Collection

Four venous blood samples (6ml each) were taken using standard draw procedure by the RMW at randomisation (visit 2), post intervention (visit 3) and late pregnancy (visit 5) using the Vacuette® system:

1. 1 plain tube with clot activator for serum
2. 2 tubes with EDTA
3. 1 tube with lithium heparin

3.7.2 Processing

Samples were retrieved within 30 minutes and centrifuged for 10 minutes (Eppendorf centrifuge, 10minutes, 1400g, 4^{o}C). The serum sample was stored at room temperature for 30 minutes to allow for clotting, prior to being spun under the same conditions, followed by a further 10 minutes at 2500g.

Up to 20 aliquots were prepared for freezing from the EDTA and lithium heparin samples and ≤10 for the serum, all of 250ul. The buffy coat was removed with a 3ml Pasteur pipette, transferred to a microtube and spun for an additional 10 minutes at 1400g.

Barcoded samples were scanned onto the study database prior to freezing at -80^{o}C and remained on ice until such time.

3.7.3 Biochemistry

3.7.3.1 Biomarker selection

The choice of biomarkers analysed in the pilot study was made upon a review of the current evidence discussed in section 1.3.3.2 Mechanisms of insulin resistance (page 42).

In this thesis biomarkers implicated in the pathogenesis of GDM which also provide an indirect measure of IR and lipid metabolism were analysed: plasma insulin, plasma total cholesterol, triglycerides, high-density lipoprotein (HDL-c), low-density lipoprotein (LDL-c) and high-sensitivity C-reactive protein (hs-CRP). Additional calculations were made for very-low-density lipoprotein (VLDL) and the following ratios; cholesterol: HDL and LDL: HDL. Because of the high cost of directly measuring HDL and LDL, the surrogate markers HDL-C and LDL-c were used, representing cholesterol associated with apolipoprotein A1/HDL particles.

Furthermore we explored to predictive ability of more novel biomarkers for GDM which have been used in pregnancy studies and reflect a range of inflammatory pathways, adipose tissue function, hepatic fat accumulation and vascular dysfunction (Savvidou, Nelson et al. 2010, Ferreira, Rezende et al. 2011, Lacroix, Battista et al. 2013).

In total **16 biomarkers** were measured in this thesis as listed below and 18 reported including the two ratios cholesterol:HDL and LDL:HDL (Table 9).

Table 9 Biomarkers measured in the UPBEAT pilot study

Insulin (mU/l)	Fructosamine (umol/l)
Cholesterol (mmol/l)	ALT (U/L)
Triglycerides (mmol/l)	AST (U/L)
HDL (mmol/l)	Ferritin (ng/ml)
LDL (mmol/l)	Adiponectin (μg/ml)
VLDL (mmol/l)	tPA (ng/ml)
Cholesterol:HDL	IL-6 (pg/ml)
LDL:HDL	Leptin (pg/ml)
CRP (mg/l)	Visfatin (ng/ml)

To the best of our knowledge no other work exploring the prediction of GDM using these biomarkers in a specifically obese pregnant population has been performed.

3.7.3.2 Analysis

Plasma total cholesterol, HDL-cholesterol triglycerides, ALT, AST, hs-CRP, fructosamine (c311, Roche Diagnostics, Burgess Hill, UK) and ferritin (elecsys 2010, Roche Diagnostics, Burgess Hill, UK) were measured on clinically validated automated platforms using the manufacturers' quality controls and calibration materials. Coefficients of variation (CVs) were <6%.

Plasma insulin was measured by an enzyme linked immunosorbent assay (ELISA) (Mercodia, Uppsala, Sweden) that does not cross-react with proinsulin, with an interassay CV <7%.

Baseline plasma adiponectin, IL-6, leptin (R&D Systems, Abingdon, U.K.), t-PA (Stago, Theale, UK) and visfatin (Phoenix peptide, Karlsruhe, Germany) were measured by enzyme-linked immunosorbent assay. These methods had inter-assay CV's <10%.

All analyses were performed on previously unthawed EDTA and serum samples at the British Heart Foundation Laboratory at the University of Glasgow.

Plasma NEFA and fatty acids were measured on samples obtained after fasting for 12 hours at 27^{+0}-28^{+6} week's gestation in the KCL Division of Nutritional Sciences.

NEFA was measured on a clinically validated automated platform (Clinical Analyser ILab 650, Instrumentation Laboratories, Warrington, UK) using the Randox (FA115) kit with quality control (QC) performed after each 60 sample batch at the upper and lower range of the assay with the following CVs:

NEFA
QC1 target 1.24 mmol/l, %CV 0.95
QC2 target 0.58 mmol/l, %CV 0.97

Plasma fatty acids were measured by gas-liquid Chromatography (GC) (Bondia, Castellote et al. 1994). Esterified and non-esterified fatty acid methyl esters (FAMEs) were analysed using the one-step transesterification direct method (Lepage and Roy 1984). Main fatty acids peaks (C16:0, C18:0, C18:1(n-9), C18:2(n-6), C18:3(n-3), and C20:4(n-6) were recognised by referring to standard retention times (Sigma-Aldrich Co. Ltd, Gillingham, UK). C20:5(n-3) and C22:6(n-3) were determined by cod liver oil FAME standards (Sigma-Aldrich Co. Ltd (Gillingham, UK). The remaining FAs were measured by GC mass spectrophotometry (GC-MS). Each plasma FA concentration was determined as the area under the peak matched with the known standard (Sun, Ma et al. 2007).

3.8 Statistical analysis

3.8.1 Analysis of the UPBEAT pilot data

Data are presented by GDM status (GDM versus non GDM) in keeping with the theme of this thesis and where relevant by randomised treatment (control versus intervention) for the following categories: demography, maternal anthropometry, biomarker and NEFA analysis with baseline measurements taken at randomisation (15^{+0}-17^{+6} weeks').

It is important to note that analysis comparing the overall difference for each biomarker as shown in Table 18 included data from all three visits. In contrast, the development of a prediction model for GDM using biochemical and clinical variables, discussed in 5.5 Prediction model for GDM (page 128), included data obtained at randomisation only (16^{+0}-18^{+6}).

Log transformations were made for all biochemical variables following standard distribution checks and parametric tests, described in standard methodology, were used where appropriate (Altman 1990).

The effect of randomised treatment was estimated by random effects Generalised Least Squares (GLS) multiple regression clustering by patient with robust standard errors and adjustment for visit and GDM status. GLS is a technique for estimating the unknown parameters in a linear regression model. The GLS is applied when the variance of the observations are unequal, or when there is a certain degree of correlation between observations as would occur during time series analyses. This would include measurements recorded over the course of pregnancy as in UPBEAT. For a comparison between women who developed GDM and those who did not, the same method was used but with adjustment for visit and randomised intervention (Arellano 1987).

Differences between patient groups are reported as arithmetic means (SD) with 95% confidence intervals. Statistical significance was <0.05.

3.8.2 Prediction of GDM

The analysis was exploratory with the aim of identifying potentially useful combinations of clinical and biochemical predictors of maternal GDM with an aim to develop a multivariable prediction model. Therefore, potentially useful biomarkers were not excluded and no adjustment was made for multiple testing. Standard distributional checks (BoxCox regression and Normal distribution plots) were carried out, and separate decisions made on the appropriate transformation. Based on these findings, log transformation was made for all biochemical variables. Differences between patient groups are reported as geometric means and ratios of geometric means, with 95% confidence intervals.

Customised birth centiles were calculated using the GROW calculator (version 6.7) (http://www.gestation.net/birthweight_centiles/birthweight_centiles.htm) which adjusts for gestational age, sex, maternal BMI, height and ethnicity, for analysis of the predictive power of the biomarkers on secondary clinical outcomes SGA, LGA and macrosomia (Gardosi, Chang et al. 1992).

The association of clinical indicators with GDM was established using linear or logistic regression as appropriate, with robust standard errors. Biochemical indicators were assessed as predictors of GDM, adjusting for significant clinical indicators. Following univariate analysis, those variables which were identified as independent predictors of GDM were included in the model.

Table 10 Summary of clinical and biochemical variables used in the development of a prediction model for GDM

Clinical Measures		Biomarkers	
Age (years)	Smoking status	Insulin (mU/l)	Fructosamine (umol/l)
Height (m)	Material circumference (cm) Waist, mid-arm, hip, thigh	Cholesterol (mmol/l)	ALT (U/L)
Weight (kg)	Maternal skinfolds (mm) Triceps, biceps, subscapular, suprailiac, total sum	Triglycerides (mmol/l)	AST (U/L)
BMI (kg/m^2)		HDL (mmol/l)	Ferritin (ng/ml)

Parity ≥2	LDL (mmol/l)	Adiponectin (μg/ml)
Ethnicity	VLDL (mmol/l)	tPA (ng/ml)
Deprivation score	Cholesterol:HDL	IL-6 (pg/ml)
Systolic BP (mmHg)	LDL:HDL	Leptin (pg/ml)
Diastolic BP (mmHg)	CRP (mg/l)	Visfatin (ng/ml)

Internal validation using discrimination to assess the overall performance of the markers as predictors of GDM was assessed by comparison of ROC areas. Calibration was not performed. Where necessary, composite predictors were derived using multiple logistic regression.

All data analysis was carried out using the statistical package Stata, version 11.2 (StataCorp, College Station, Texas).

4 METHODS: IMPROVING GLYCAEMIC PROFILES IN OBESE PREGNANCIES (IGPOP)

The IG-POP study was designed to evaluate the glycaemic response of two nutritional supplements A and B, developed by Abbott Nutrition with the aim of blunting PPG in obese pregnant women (OP). This was the first study to trial palatability of the products in pregnant women and to test this hypothesis.

Three supplements were initially developed, A, B and C with a control D. However following concerns regarding palatability in the early stage of phase 1, C was excluded from the study. For this thesis, only A, B and D will be discussed which were all banana flavoured.

For stage 2, B was redeveloped due to issues of excess sediment and the composition of D was modified based on stage 1 results with an increase in total fat content to match B. Both supplements were therefore assigned new identification codes (Table 11).

All study visits took place in the dedicated clinical research facility (CRF) at St. Thomas' Hospital, London with a member of the IGPOP team, consisting of a clinical research fellow and part-time dietician.

Ethical approval: NHS approval was granted by the Riverside South West Research Ethics Committee (Reference number 12/LO/0307).

4.1 Dietary composition of nutritional supplements

The detailed macro and micronutrient composition of all products used in the IG-POP study are summarised in Table 11.

Supplements were produced by Abbott Nutrition, Columbus, Ohio and exported to the UK in 8oz (237ml) ready-to-drink cartons.

Table 11 Detailed macro and micro nutrient composition of products used in IGPOP stages 1 and 2

	STAGE 1			STAGE 2	
	12501RF(A) Low fat	**12500RF(B)** High fat	**12506RF(D)** Control	**12539RF(B)** Intervention	**12551RF(D)** Control
Volume of serving (oz)	8	4	8	4	4
Frequency of serving	-	-	-	Twice a day	Twice a day
Macronutrients per 8oz					
Calories (kcal)	149	303	152	303	303
Protein (g)	12	14	9.5	14	14
% calories from protein	32.3	18.5	25	18.5	18.5
Total Fat (g)	0.5	7	2	7	7
% calories from fat	3.0	20.8	11.8	20.8	20.8
Saturated fat (g)	-	-	-	-	-
Trans fat (g)	-	-	-	-	-
DHA (mg)	50	100	50	100	100
Carbohydrate (g)	24	46	24	46	46
% of calories CHO	64.6	60.7	63.2	60.7	60.7
MD 9-16 (g)	1.9	3.7	-	-	-
% of CHO	8	8	-	8.4	-
Isomaltulose (g)	16.3	31.3	-	18	-
% of CHO	68	68	-	72	-
FOS (g)	0.8	1.6	-	0.9	-
% of CHO	3.5	3.5	-	3.7	-
Fibersol (g)	3.7	7.1	-	4.1	-
% of CHO	15.5	15.5	-	16.32	-
Lactose (g)	1.2	2.3	-	-	39
% of CHO	5	5	-	-	85
Sucrose (g)	-	-	-	-	6.9
% of CHO	-	-	-	-	15
Fiber (g)	-	-	-	3.1	0
Sugars (g)	-	-	-	33	46
Maltodextrin (DE20-23)	-	-	24	-	-
% of CHO	-	-	100	-	-

Micronutrients per 8oz	**12501RF(A)** Low fat	**12500RF(B)** High fat	**12506RF(D)** Control	**12539RF(B)** Intervention	**12551RF(D)** Control
Vitamin A (IU)	405	405	-	405	405
(mcg)	122	122	-	122	122
Vitamin C (mg)	70	70	-	70	70
Vitamin D (IU)	200	200	-	200	200
(mcg)	5	5	-	5	5
Vitamin E (IU)	14.2	14.2	-	14.2	14.2
(mg)	9.5	9.5	-	9.5	9.5
Vitamin K (mcg)	16.2	16.2	-	16.2	16.2
Calcium (mg)	500	500	-	500	500
Iron (mg)	6.1	6.1	-	6.1	6.1
Thiamin (B1)(mg)	0.8	0.8	-	0.8	0.8
Riboflavin (B2)(mg)	0.8	0.8	-	0.8	0.8
Niacin (mg)	5.2	5.2	-	5.2	5.2
Vitamin B6 (mg)	1	1	-	1	1
Folic acid (mcg)	300	300	-	300	300
Vitamin B12 (mcg)	2.2	2.2	-	2.2	2.2
Biotin (mcg)	4.4	4.4	-	4.4	4.4
Pantothenic acid (mg)	3	3	-	3	3
Sodium (mg)	102	102	-	102	102
Potassium (mg)	419	419	-	419	419

Phosphorus (mg)	476	476	-	476	476
Iodine (mcg)	22	22	-	22	22
Magnesium (mg)	115	115	-	115	115
Zinc (mg)	10	10	-	10	10
Selenium (mcg)	32	32	-	32	32
Copper (mg)	0.5	0.5	-	0.5	0.5
Manganese (mg)	0.6	0.6	-	0.6	0.6
Comium (mcg)	30	30	-	30	30
Chloride (mg)	254	254	-	254	254
Choline (mg)	120	120	-	120	120

4.1.1 Stage 2

The macronutrient composition was equivalent for both supplements in stage two but the concentration of CHO sub-groups, known to affect absorption differed significantly, with the intervention product composed of more slow-digesting, low-GI carbohydrates (SG-LGI) (Table 12). Supplements were produced in similar 8oz (237ml) cartons however subjects were required to consume 4oz twice a day with breakfast and as an afternoon snack at 1500hr to make up the total daily dose; marked measuring cups were provided for ease. Table 11 details the breakdown of CHO content for both supplements per 24 hours of each test day.

Table 12 Detailed carbohydrate breakdown of supplements used in stage 2 per 24 hours for each test day

Carton size 8oz* (total daily dose)	**Intervention (12539RF) B**	**Control (12551RF) D**
Glycaemic load (GL)	730	1124
Calories (Kcal)	303	303
Total fat (g)	7	7
% calories from fat	20.8	20.8
Protein (g)	14	14
% calories from protein	18.5	18.5
Carbohydrate (CHO) (g)	46	46
% calories from CHO	60.7	60.7
Rapid digesting (%)	8.4	100
Slow digesting (%)	71.6	0
Resistant starch (%)	16.3	0
Indigestible fibre (%)	3.7	0

*one whole 8oz carton was consumed over each test day in two divided 4oz servings

4.2 Data collection and database development

An online secure database was designed specifically for the IGPOP study and developed with Medscinet® (http://www.medscinet.net/igpop). This ensured paper free data collection since everything was recorded directly onto the database to minimise transcription error.

Randomisation was programmed into the database software using the SQL Server randomization function that randomly selects a drink each time. Therefore the final supplement drink was not random since simple elimination was adopted following each test.

All data including CGMS outputs was backed up in a secure encrypted file on the university network.

4.3 Stage 1

Stage 1 was single blinded and sub-divided into 1a and 1b.

In stage 1a, the glycaemic response of A, B and D was evaluated in four categories of women (n=10 per group) following a standard meal tolerance test (MTT) with capillary blood glucose (CBG) measured at 9 defined time points, up to 240 minutes. Each woman was required to attend the CRF on 3 separate occasions with a minimum 2 day washout period between visits.

1. Lean non-pregnant [LP] (BMI ≥18.5-≤24.9kg/m^2)
2. Obese non-pregnant [ONP] (BMI ≥30kg/m^2)
3. Lean pregnant [LP] (BMI ≥18.5-≤24.9kg/m^2 – self reported pre-pregnancy BMI)
4. Obese pregnant [OP] (BMI ≥30kg/m^2 -self reported pre-pregnancy BMI)

In stage 1b, the GI of the drink with the lowest AUC from stage 1a was calculated. Ten healthy, lean women (BMI ≥18.5-≤24.9kg/m^2) attended the CRF on two separate occasions for a MTT for the test drink and a 50g glucose

standard. Standard GI methodology was followed and is detailed in the protocol (Brouns, Bjorck et al. 2005).

4.3.1 Recruitment

Non-pregnant participants were recruited through a local advert distributed to all students and staff of King's College London's via the university email service.

Pregnant women were approached by a member of the IGPOP team at routine antenatal and fetal scanning clinics at Guy's and St Thomas' NHS Foundation Trust (GSTT) at 12 or 20 weeks gestation. Pre-pregnancy BMI was ascertained in advance from data held on the Terranova® heath ware system uploaded by community midwives at the 1st antenatal visit.

Following the initial contact a patient information sheet was provided to all interested women and after a minimum of 48 hours, a follow-up telephone call made to discuss participation and arrange the first visit if verbal consent was given. Written consent was obtained by all women at the 1st face to face appointment with the study team.

Participants were offered complimentary taxi transport for all appointments.

Inclusion criteria for stages 1a and 1b

- Minimum age 18-40 years including age at conception
- Able to understand and write English (to enable written informed consent including pregnancy testing for those in the non-pregnant groups in 1a and 1b)
- Able to attend CRF on at least four occasions for stage 1a and two for stage 1b
- Appropriate BMI for each category (early pregnancy BMI, confirmed from antenatal booking notes for obese and lean subjects)
- 24-28 weeks' gestation at first study visit for those in the pregnant groups (LP & OP)

Exclusion criteria for stages 1a and 1b

- Multiple pregnancy
- Suffering from any medical condition known to independently influence weight, body composition or biochemistry including, but not limited to, thyroid disturbances, lupus, autoimmune polyglandular syndrome (APS), treated hypertension, Cushing's disease, cancer, HIV/AIDS, renal disease, liver disease, familial hyperlipidaemia, past history of diabetes (Type 1, 2, and gestational) and polycystic ovary syndrome (PCOS).
- Gestational diabetes in index pregnancy diagnosed by local clinical trust guidelines (fasting blood glucose ≥6.1mmol or 2 hour glucose ≥7.8mmol following a 75g OGTT)**.**
- Lactose, gluten or wheat intolerant
- Receiving treatment or currently suffering from an untreated eating disorder
- Currently following a modified diet

4.3.2 Sample size

Sample size in stage 1 complies with standard published GI testing methodology, which recommends a sample size of ten subjects to generate clinically useful GI values with 80% power at a level of $p<0.05$ (two tailed) (Brouns, Bjorck et al. 2005).

4.3.3 Protocol

Stage 1a

Preparation and arrival

Women attended the CRF at 0830hr fasted for all 3 (instead of the original 4) visits, following the withdrawal of drink C.

Detailed instructions for study day preparation were sent in advance (Appendix 1: IGPOP Supplementary documents, page 191). The overnight fast commenced at 2200hr following a 30-50g CHO meal (example meals were included) and avoidance of strenuous activity and caffeine was advised.

Height was measured using the free standing Leicester Measure® stadiometer and weight with the Marsden® digital scales in light clothing, without footwear. Non-pregnant women were excluded if BMI did not meet the inclusion criteria for the group and for pregnant women, pre-pregnancy BMI was re-confirmed from antenatal notes.

Blood pressure was recorded using an appropriate cuff with the Microlife® BP3BT0 automated device, validated for use in pregnancy. A medical and drug history including vitamin supplements was taken and urinary pregnancy testing performed to confirm status of the non-pregnant women.

Supplement Drinks

For the MTT, each supplement drink provided the equivalent of 46g of carbohydrate in a total volume of 500ml. Owing to the different CHO composition of the supplements per 8oz carton (237ml) (Table 11), preparation was slightly different to ensure standardisation:

1. Drink A-x2 8oz cartons (474ml) mixed with water to a final volume of 500ml
2. Drink B-x1 8oz carton (237ml) mixed with water to a final volume of 500ml
3. Drink D-x2 8oz cartons (474ml) mixed with water to a final volume of 500ml

Following baseline measurement of CBG at 0 minutes, the drink was consumed over a maximum of 5 minutes and subjects were free to drink water over the next 240 minutes. If the 0min CBG test was ≥6.1mmol, in keeping with impaired fasting glycaemia (IFG), subjects were excluded from the study.

CBG Measurements

Capillary blood glucose was measured at 9 time points using a calibrated Abbott FreeStyle® Navigator glucometer assigned to each women for the study.

Timing of CBG measurements: 0, 15, 30, 45, 60, 90, 120, 180 & 240 minutes

Completion of test

During the test participants were asked to complete an online palatability questionnaire. At the end, concerns regarding any aspect of the test were discussed and participants were given a snack prior to leaving the CRF.

Primary and secondary outcomes

The primary outcome of stage 1a was capillary blood glucose concentration measured at 9 time points to determine AUC for each drink in each of the 4 categories of obese and lean pregnant women. The secondary outcome was to identify any issues regarding palatability for supplements A and B particularly in the pregnant groups to inform the study design of stage 2.

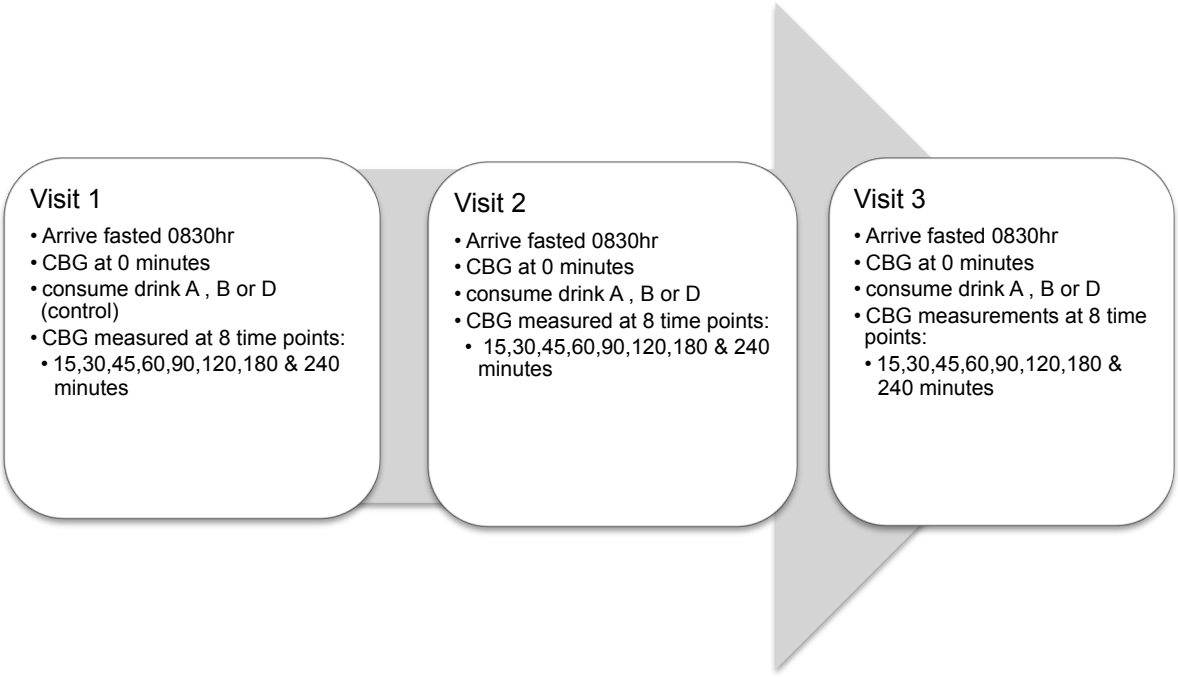


Figure 14 Flow chart for stage 1a for the 4 different groups (total n=40)

Stage 1b

Following the analysis of stage 1a data, a decision was made by the research team with Abbott Nutrition to use drink B in stage 2. Therefore in stage 1b, the GI value of drink B was determined in 10 healthy women.

The recruitment process and inclusion/exclusion criteria were identical to the selection of non-pregnant women in 1a who were invited to participate again.

Standard published methodology for GI testing was used (Brouns, Bjorck et al. 2005). For this, a minimum of 10 subjects with no significant past medical history are required to consume a 50g CHO serving of the test food and 50g of glucose as the standard. Serial CBG are measured over 120 minutes to calculate the AUC which is then expressed as a percentage of the mean AUC for the standard (Wolever, Jenkins et al. 1991).

Preparation and arrival

This was the same as for stage 1a. Participants were required to attend the CRF for 2 test days with a minimum 2 day washout.

To provide 50g CHO of supplement drink and standard, the following volumes were given:

1. Drink B: 257ml
2. Standard: 273ml of Lucozade Original® (70kcal/100ml)

After the baseline CBG was taken (0min), supplements were consumed within 5 minutes and once again water was permitted.

CBG Measurements

Capillary blood glucose was measured at 7 time points up to 2 hours using a calibrated Abbott FreeStyle® Navigator glucometer assigned to each women for the study.

Timing of CBG measurements: 0, 15, 30, 45, 60, 90 and 120 minutes

Completion of test

A snack was given prior to going home and any concerns addressed.

Primary outcome

The primary outcome measure was glucose AUC following 50g CHO of drink B and the standard (Lucozade Original®).

4.4 Stage 2

Stage 2 was a single blinded randomised crossover design in obese pregnant women. The aim was to evaluate the immediate and extended effects on glucose and insulin concentrations, following consumption of the control or intervention (drink B) as part of a controlled diet using CGMS. The study was divided into three distinct 48 hour periods (intervention/control, washout and intervention/control) and subjects were required to attend the CRF twice, on the 1st day of each test phase.

4.4.1 Recruitment

Recruitment was the same as for pregnant women in stage 1. As these women were naive to CGMS, all were invited to attend a screening visit to assess competency using the system and offered a trial wearing the sensor at home.

Participants were offered complimentary taxi transport for all appointments.

Inclusion criteria

- Singleton pregnancy with minimum age at conception of 18-40 years
- Early-pregnancy BMI ≥ 30 kg/m^2
- 24^{+0} -28^{+6} weeks' gestation at 1st study visit
- Able to understand and write English and give informed consent
- Able to attend the CRF on a Thursday and consecutive Monday according to the protocol

Exclusion criteria

Exclusion criteria were the same as those used in stage 1. Following randomisation, participants were excluded if GDM was diagnosed in the time window between consent and the 1st test day or suspected during the course of the study. In this circumstance, women were referred to the local diabetes department for a 75g OGTT.

4.4.2 Sample Size

The study was a randomised crossover design of intervention versus control in obese pregnant women. In the protocol development phase, the power calculation detailed

below was based on the assumption that the analysis would be performed using the mean glucose concentrations derived from CGMS (AUC).

Preliminary analysis of raw CGMS data by Dr Garcia at Seplin Solutions revealed high intra-day variability with 13 women randomised to sequence 1 (intervention/control) and 9 to sequence 2 (control/intervention), which was shown to have introduced substantial bias in using the mean CGMS data. To correct for this, a random subsample of 16 patients (n=8 for sequences 1 and 2) was selected for analysis using linear regression with mixed modelling. This is expanded in section 4.5.2 Statistical analysis for IGPOP page 110.

The findings of previous studies in obese pregnant women informed the power calculation. Harmon and Yogev, found the SD for glucose measurements in obese pregnant women to be 13 or 12 mg/dl respectively (Yogev, Ben-Haroush et al. 2004, Harmon, Gerard et al. 2011). It is felt that a difference of 0.5mmol/l (9mg/dl) in glucose measurement when comparing standard diet versus intervention diet is likely to be clinically and scientifically important. This 9 mg/dl is also similar to the differences found under various conditions between obese and normal weight women (Harmon, Gerard et al. 2011). We know of no robust data on correlations between repeated measures of serum glucose in diabetic subjects and therefore considered a range of plausible values (correlation coefficients: 0.1, 0.3, 0.5, and 0.7), with a mean of 9mg/dl and SD of 12.5mg/dl based on values (12 and 13) from the two papers.

Correlation coefficient (r)	0.1	0.1	0.1	0.3	0.3	0.3	0.5	0.5	0.5	0.7	0.7	0.7
Sample size (n)	15	20	22	15	20	22	15	20	22	15	20	22
Power (%)	54.7	67.0	71.7	65.4	77.7	81.4	79.6	89.6	92.2	95.0	98.6	99.2

Table 13 Summary of power calculation for Stage 2

Previous studies using a similar LGI product by Abbott Nutrition, known as Glucerna® in T2DM (n=15 and mean participant BMI 30kg/m^2), demonstrated significant improvements to postprandial glycaemia (data courtesy, Abbott Nutrition). This suggested that a study of 22 subjects would be adequate even for very low correlation coefficients (e.g. < 0.5 commonly found in biochemical

measurements, repeated over short time intervals). Taking into consideration participant drop out and incomplete data we aimed to recruit 25 women.

4.4.3 Protocol

A flow chat outlining the key features of stage 2 is shown below (Figure 15).

Pre-study visit

All women were required to have a visit 1-2 weeks' prior to the study with the clinical research fellow. The aim was to give women the opportunity to handle the CGMS device and learn how to perform a calibration with a CBG test using the in-built glucometer. This was also an opportunity for the women to ask any questions about the sensor and explain the relevant additional features, particularly the alarm systems. Information sheets on how to care for the device including precautionary everyday measures e.g. around water, were also provided. By the end of the appointment, a joint decision based on competency and confidence was made regarding progression to the study.

Menu options for the controlled diet were discussed and meal choices selected. All dietary preferences and allergies were documented on the database.

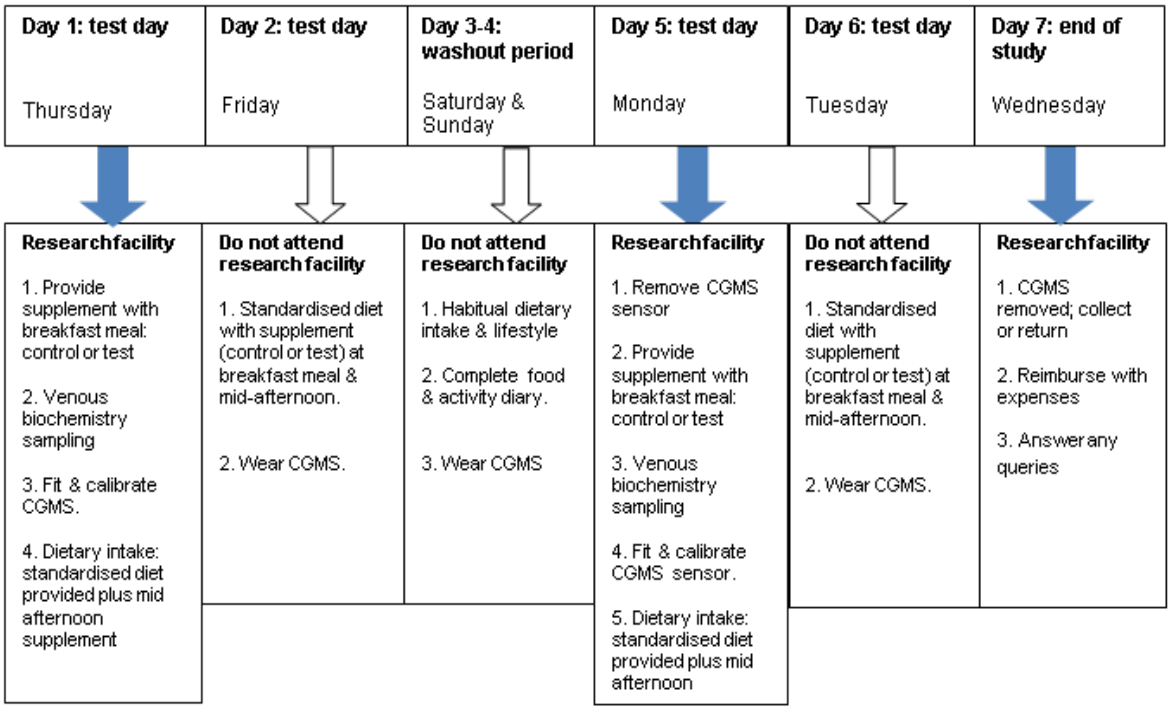


Figure 15 Summary of IGPOP Stage 2 by individual day

CGMS: Abbott FreeStyle® Navigator

The Abbott FreeStyle® Navigator was used which has 3 components (Table 16):

1. The subcutaneous sensor coated with glucose oxidase (GOx).
2. The transmitter connecting to the sensor and worn over the skin.
3. The receiver unit, which links wirelessly to the transmitter using bluetooth™ technology within a 3m range.

Interstitial glucose concentration is measured via a concentration-dependent current generated by the movement of electrons from an oxidation reaction between ISF glucose and GOx. In the FreeStyle® Navigator, glucose oxidase is cross-linked with an osmium mediator to directly capture the electrons from the initial oxidation reaction in contrast to other commercially available devices which measure electron movement from the indirect production of hydrogen peroxide. The addition of this mediator enables the sensor to operate at very low voltage potentials (40mV) thus preventing interference from other ISF constituents (Oliver, Toumazou et al. 2009).

The system requires calibration with CBG using the in-built glucometer at five specific time points: 1, 2, 10, 24 and 72 hours. The receiver alarms prompts the user to perform a CBG test on each occasion and only begins to record glucose following a successful 1 hour calibration initially.

The system allows for meal markers to be set by the user for analysis of post-prandial glucose changes. Women were taught how to use this feature at the pre-study visit and encouraged to demonstrate this again under supervision again during CRF test days.

The sensor is approximately the size of a penny and made from a plastic material. A spring-loaded device was used to insert the sensor under aseptic technique into the subcutaneous fat to a depth of approximately 5mm and width <1mm. Although studies have demonstrated no difference in the performance and accuracy of the system when sited on the upper arm or abdomen, all women preferred the upper arm. Data collected from participants was uploaded from the receiver via Bluetooth™ onto the CoPilot® Health Management System designed specifically for the FreeStyle® Navigator.

a) b)

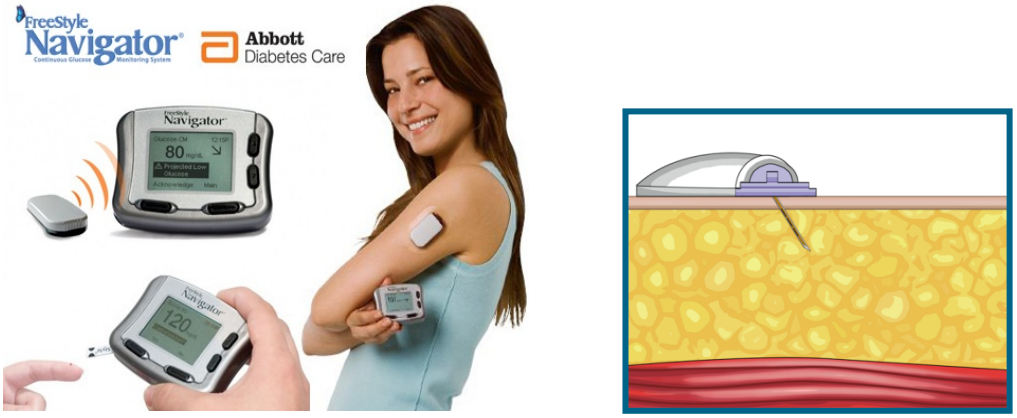


Figure 16 a) The Abbott FreeStyle® Navigator system components and b) Subcutaneous position of the CGMS sensor

Dietary intake

A standardised diet with a low residue and medium to high dietary GI reflecting the "average UK diet" (National Diet and Nutrition Survey 2008/2009, Food Standards

Agency:http://tna.europarchive.org/20110116113217/http://www.food.gov.uk/multimedia/pdfs/publication/ndnsreport0809.pdf) (Aston, Gambell et al. 2008) was provided for the two 48 hour test periods (days 1 and 2 [Thursday, Friday] and day 5 and 6 [Monday, Tuesday]). On days 1 and 5, breakfast and lunch were provided in the CRF with the remaining food and supplement drinks measured out by research staff and packed to take home. Women were advised to eat at similar times on each test day and asked to return all empty drink cartons.

A half carton (4oz) of the intervention/control drink was to be consumed twice a day and a marked measuring cup was provided:

1. At breakfast (0830hr)-with cereal instead of milk
2. As an afternoon snack at 1500hr

A copy of the menu choices developed by a research dietician is included in the appendix (12.5 Meal Choices For IGPOP Stage 2, page 208). An example menu for the CRF test days with composition, calculated using standard food tables and WISP dietary software is shown below (Table 14). Unlimited water and black tea/coffee without sugar were permitted and a daily milk allowance was included.

Table 14 Example of Menu A consumed on CRF test days (days 1 and 5). Alternative food choices are given in the appendix

MENU A-Day 1 & 5	Energy (Kcal)	Total CHO (g)	Total sugars (g)	Total protein (g)	Total fat (g)	Total fibre (g)	GI*
09.30 Breakfast							
Cornflakes (17g variety pack)	61	14	1	2	0	1	93
Test or control supplement	152	23	17	7	4	2	27*
Meal total	**212**	**37**	**18**	**9**	**4**	**2**	
13.00 Lunch							
Branston macaroni cheese (395g)	376	55	6	13	11	2	35
Cheddar cheese (60g)	219	0	0	14	18	0	34
Nature's Finest Tropical Fruit Salad pot (in juice) 113g	67	14	13	3	0	1	50
Meal total	**662**	**69**	**19**	**30**	**29**	**2**	
15.00 AFTERNOON							
Test or control supplement	152	23	17	7	4	2	27
18.30 DINNER							
Sainsbury's spinach and ricotta cannelloni (400g)	595	55	12	20	31	7	
Ambrosia chocolate custard (150g)	171	28	22	5	4	1	38
Meal total	**766**	**83**	**34**	**25**	**35**	**8**	
20.30 SUPPER & MISC							
Philadelphia tub (35g) snack	55	1	1	3	4	0	34
Philadelphia tub (35g) snack	55	1	1	3	4	0	34

Poppy and sesame thin crackers x 4	80	10	0	2	4	1	65
Sainsbury's grape pack (80g)	53	12	12	0	0	1	46
Meal total	**243**	**25**	**16**	**8**	**12**	**2**	
Meal total excluding supplements	**1883**	**214**	**86**	**71**	**80**	**14**	
Total	2035	237	102	78	84	15	

Dietary data were generated using the WISP dietary data software

*Estimation of GI. For test supplement, GI given is calculated form Stage 1b IGPOP.

Preparation and arrival

Preparation and arrival for study days was identical to stage 1. A participant's handbook was designed which contained "handy hints" on how to calibrate the sensor, manage alarms or warning symbols and reconnect with the transmitter if connection was lost (i.e. >3m range is exceeded) (hard copy enclosed in appendix). It also included healthy dietary advice, a food diary to record all intake during the study and full contact details of the research group.

Before each study day, the date and time on each paired receiver/transmitter were checked, all batteries replaced and any previous glucose data from preceding participants uploaded onto Co-pilot® and deleted from the receiver.

The sensor was inserted on day 1 (Thursday) by the research team who encouraged all women to perform the 1 hour calibration themselves under supervision to ensure correct initialisation. On day 5 (Monday) the old sensor containing data from the 1st test phase and weekend habitual period was removed on arrival and a new one sited. As a result, there was a time gap of missing data prior to the 1st 1 hour calibration of the new sensor. During the hour wait before calibration, a venous cannula was inserted in the antecubital vein and the randomised drink prepared.

Meal Test

Blood samples were taken every fifteen minutes from 0-210 minutes and glucose was measured on the YSI 2300 STAT Plus™ glucose and lactate analyser (Yellow Springs Instrument, Ohio, USA) in the CRF on each occasion prior to processing.

Sample time points were: 0, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195 and 210 minutes.

Following confirmation that the sensor was working correctly, the 0min blood sample was taken and the intervention/control drink consumed within 5 minutes.

During the meal test women were shown how to complete the food diary and any queries were discussed. The 2 hour sensor calibration was also performed in the CRF and all sensors checked to be working correctly at regular intervals.

Completion of test

The pre-measured food was provided with the appropriate number of supplements prior to departure. The research dietician explained the menus again, stressing the importance of adherence to the controlled diet until midnight on the Friday (day 2) when habitual diet could be followed until fasting on Sunday night in preparation for the next study day on Monday; non-adherence to the controlled diet was identifiable from CGMS readings and data for such subjects was excluded from analysis. All consumables such as glucose test strips, lancets and a sharps bin plus a CGMS quick reference guide including contact telephone numbers were provided.

Primary and secondary outcomes

The primary outcome of stage 2 was measurement of plasma glucose, insulin and C-peptide concentrations following ingestion of a SD-LGI drink to evaluate the acute response (up to 210 minutes) in a controlled environment compared to a drink of rapidly digesting CHO.

Secondary outcomes included measurement of the prolonged effects of the SD-LGI drink on glucose concentrations (up to 48 hours) and evaluation of habitual glucose profiles in obese pregnant women at 24-28 weeks', using CGMS for both.

4.4.4 Blood samples

Fifteen venous blood samples were taken on each of the two study days at the CRF to provide an immediate measure of the plasma glucose concentration, and a sample for later measurement of C-peptide, insulin, NEFA and triglyceride concentrations. Insulin, C-peptide and glucose were measured at all 15 time points, and TG and NEFA at 5 time points: 0, 60, 120, 180 and 210 minutes.

4.4.4.1 Collection and processing

Using aseptic technique, an initial 1ml of blood representing the "dead space" within the cannula was aspirated and discarded. A further 2ml was then aspirated for analysis and divided into 3 tubes:

1. 0.75ml-EDTA (red) for TG and NEFA
2. 0.5ml-Lithium heparin (orange) for YSI glucose assay.
3. 0.75ml-Sodium heparin plasma (yellow) for insulin and C-peptide

Lithium heparin samples were analysed directly from whole blood at room temperature on the YSI analyser. Regular maintenance of the instrument was performed by the clinical research fellow in accordance with manufacturer's guidelines, following appropriate training.

The remaining samples were processed within 5 minutes and centrifuged for 10 minutes (Eppendorf centrifuge, 10minutes, 1400g, 4^{o}C). Single aliquots were prepared and barcoded samples were scanned onto the study database prior to freezing at -80^{o}C.

4.4.4.2 Analysis

Plasma TG and NEFA were measured on a clinically validated automated platform (Clinical Analyser ILab 650, Instrumentation Laboratories, Warrington, UK) using the IL Triglyceride and Randox (FA115) kits in the KCL Division of Nutritional Sciences. Quality control (QC) was performed after each 60 sample batch at the upper and lower range of the assay with the following CVs:

Triglycerides	NEFA
QC1 target 1.10 mmol/l, %CV 2.39	QC1 target 1.24 mmol/l, %CV 0.95
QC2 target 3.00 mmol/l, %CV 1.12	QC2 target 0.58 mmol/l, %CV 0.97

Plasma insulin and C-peptide were measured using manual ELISA kits (Mercodia, Uppsala, Sweden) at the MS2 SAS Peptide Hormone Laboratory, Royal Surrey County Hospital, Guildford, UK. All standards, controls and samples were assayed in duplicate and for any sample yielding a result above the top standard, repeated

analysis in dilution using the zero standard from the appropriate kit was re-run. All controls were performed at both front and back of each ELISA plate.

4.4.5 Criteria for downloading and cleaning CGMS data

CGMS data was uploaded into the Abbott software, Co-pilot® (version 3.0) and exported into Microsoft Excel®, immediately following sensor removal at the last visit.

A strategy for reviewing and cleaning data using clinically relevant and accepted methods, was agreed by the research team, which included Dr Helen Murphy (University of Cambridge), a consultant diabetologist with extensive experience of using CGMS, particularly the FreeStyle® Navigator, in pregnancy. Marianna Nodale, a research mathematician with an interest in CGMS from Dr Murphy's group, provided additional support. A summary of the consensus is detailed below:

Table 15 Process used for cleaning CGMS data for analysis

Two clinicians (including myself), blinded to the randomisation order, independently reviewed and later crosschecked all CGMS data. The following points were reviewed:
Missing time points ACTION: coded as missing data
Data out of sensor (i.e. <1.1mmol/l) and manufacturer's temperature range ACTION: data point excluded plus data within 30 minutes (i.e. 3 data points) either side
Identification of obvious erroneous results (i.e. large variability in glucose readings out of keeping with glucose trend (drop of ≥+/-2mmol) ACTION: data point excluded plus data within 30 minutes (i.e. 3 data points) either side
Sensor failure ACTION: omit data 30 minutes prior to failure and 30 minutes (i.e. 3 data points) after resumption of sensor function ACTION: data previously coded as out of sensor range and/or out of temperature range was excluded-data point excluded plus data 30 minutes either side
Hypoglycaemia ACTION: This was defined CGMS glucose **<3.3mmol/l***

Hyperglycaemia ACTION: preprandial glucose ≥5.27mmol/l, 1-hour post meal glucose ≥7.78mmol/l, 2-hour post meal ≥6.67mmol/l
Meal Markers ACTION: Meal markers at the start of meal/snack were identified from CGMS (patient meal markers). For missing meal markers, we used meal/snack times recorded in the CRF and confirmed appropriate changes in CGMS blood glucose levels which were agreed between the two project leads (RM and NP).

*A clinical definition was agreed using relevant studies and clinical guidelines for hypoglycaemia in subjects with diabetes (Gangji, Cukierman et al. 2007, Seaquist, Anderson et al. 2013)

4.5 Statistical analysis for IGPOP

4.5.1 Stage 1

Raw data extracted from the online IGPOP database was transferred into GraphPad Prism®, software for analysis (San Diego, California, USA, version 5.01 for windows). Using the mean glucose concentration (n=10 per group), the incremental area under the curve (iAUC) was calculated to adjust for differences in fasting blood glucose concentrations at baseline. One-way ANOVA test with Tukey's multiple comparison analysis, using the mean difference between iAUC of the three supplements per participant, allowed for comparison of the supplements.

4.5.2 Stage 2

Preliminary analysis using the mean CGMS value per 10 minute time point, revealed high intra-day variability (expectantly around meal times) with a non-linear structure, giving unpredictable results (GraphPad Prism®). Consequently variation was large relative to the difference in treatment groups, making it difficult to capture the differences between the groups appropriately using standardised techniques.

Linear regression with mixed modelling was therefore used and a linear mixed model (LMM) assuming a normally distributed error term was fitted to the data. LMMs are an appropriate statistical tool for repeated measures over time (West, Welch et al. 2007, West 2009). They take into account the interrelationships of the responses within subjects and are robust against dropouts. Alternative models with and without effects for period (hospital v home) and treatment were checked and

discarded as appropriate, based on the goodness of fit measures such as likelihood ratio test. When comparing LMMs with other statistical tools to model repeated data, such as Generalised Estimation Equations (GEE), it was preferable to use LMM (Gardiner, Luo et al. 2009).

Two models were plotted:

1. **Main effects model (M1)** – The predicted individual observations were plotted, allowing for straightforward overall interpretation.
2. **Full model (M2)** – This model was to gain a better understanding of the structure of the overlying data (extending from the previous model) by including the relevant interactions between variables, i.e. sequencing effects and period (hospital versus home).

Simple randomisation was adopted which did not take into account the order of the drink consumed; sequence 1=intervention/control and sequence 2=control/intervention. This led to an unbalanced allocation sequence for the 22 women included in the preliminary analysis with 13 women randomised to sequence 1 and 9 to sequence 2 which in the primary analysis was shown to have introduced substantial bias in the CGMS data (Schulz and Grimes 2002). To correct for the unbalanced design, a random subsample of 16 patients who had been allocated equally to sequence 1 and sequence 2 at the first visit was therefore selected for analysis and is presented in this thesis.

Parameter estimates of fixed effects are shown which were derived from fitting the linear mixed model to the observed data (i.e. actual CGMS data) point every ten minutes. Thus, no summary measure such as the mean is taken as a response. This is because the individual observations were modelled and therefore results are based purely on individual patient responses. Two sided t-test and estimates were obtained with 95% confidence intervals.

There was no difference when raw or cleaned CGMS data was used and results are presented for cleaned data only.

The analysis for the subsample of 16 subjects using SPSS version 19 was carried out in by Dr Llenalia Garcia Fernandez (SEPLIN statistical solutions), in close collaboration with myself.

5 RESULTS: UPBEAT

5.1 Participant characteristics

Four urban centres participated in the UPBEAT pilot study (n=183). For the purpose of this thesis, only data from three sites was included since blood sampling was not performed at King's College Hospital Foundation Trust (London).

One hundred and seventeen women were identified from three centres:

1. Guy's and St.Thomas' NHS Foundation Trust (London), n=66
2. The Southern General and Princess Royal Maternity Hospitals (Glasgow), n=24
3. The Royal Victoria Infirmary (Newcastle), n=27

Fifty-eight women were randomised to the control and 57 to the intervention. Baseline demography was equivalent between the two arms with a mean age and BMI of 30.8(5.4) years and 36.9(4.76) kg/m^2 respectively with full details listed in Appendix 2: UPBEAT pilot results, page 211).

Eleven subjects were excluded from all analysis by GDM status due to incomplete OGTT data (n=107). For evaluation of the intervention, all 117 women were included. In the main, results of comparisons by GDM status are presented with accompanying data by treatment group included in Appendix 2: UPBEAT pilot results, page 211.

Twenty-nine women were diagnosed with GDM (27.4%). Demographic and clinical characteristics of women who developed GDM compared to those who did not are summarised in Table 16. In general, women with GDM were older (p=0.001), more often of higher parity (≥2), had increased systolic and diastolic blood pressure and were more likely to be black. BMI was not significantly different between the two groups, although skinfold thicknesses were greater in women who developed GDM (triceps skinfolds, p=0.003) and total sum of skinfolds thickness (p=0.03).

Table 16 Description of subjects at baseline (15^{+0}-17^{+6} weeks' gestation) by primary outcome of GDM

Maternal Characteristic*	**No GDM (n=78)**	**GDM (n=29)**	**Comparison****	**P**
Age (years)	30.2 (5.3)	33.5 (4.4)	3.3 (1.3 to 5.3)	**0.001**
				0.03
18-25	17 (21.8%)	2 (6.9%)	--- Reference ---	
26-30	21 (26.9%)	4 (13.8%)	1.6 (0.3 to 9.9)	
31-40	26 (33.3%)	10 (34.5%)	3.3 (0.6 to 16.8)	
35^{+}	14 (17.9%)	13 (44.8%)	7.9 (1.5 to 41.0)	
Height (m)	1.65 (0.07)	1.65 (0.08)	0.00 (-0.03 to 0.03)	0.97
Weight (kg)	97.90 (15.47)	95.79	-2.11 (-7.82 to 3.60)	0.47
BMI (kg/m^2)	36.06 (4.94)	(12.38)	-0.80 (-2.52 to 0.93)	0.36
		35.27 (3.60)		
SBP (mmHg)	117.3 (9.3)	118.9 (8.1)	1.6 (-2.0 to 5.3)	0.37
DBP (mmHg)	72.3 (7.2)	74.5 (6.4)	2.2 (-0.7 to 5.0)	0.14
Circumferences (cm)				
Waist	107.4 (10.8)	107.8 (7.4)	0.4 (-3.2 to 4.1)	0.82
Mid arm	37.2 (4.0)	37.8 (4.1)	0.6 (-1.1 to 2.4)	0.48
Hip	122.9 (11.7)	120.5 (9.2)	-2.4 (-6.7 to 1.9)	0.26
Thigh	69.4 (7.6)	66.4 (9.0)	-3.0 (-6.7 to 0.7)	0.11
Skinfolds (mm)				
Triceps	31.2 (7.4)	37.4 (10.2)	6.2 (2.1 to 10.3)	**0.003**
Biceps	24.4 (7.5)	28.0 (9.5)	3.6 (-0.3 to 7.5)	0.07
Subscapular	32.2 (9.1)	36.0 (8.2)	3.8 (0.1 to 7.4)	0.04
Suprailiac	29.6 (8.3)	29.9 (8.3)	0.3 (-3.3 to 3.9)	0.87
Total	86.0 (16.7)	93.9 (16.5)	7.9 (0.8 to 15.0)	**0.03**
Ethnicity				**0.02**
White	54 (69.2%)	11 (37.9%)	--- Reference ---	
Black	21 (26.9%)	16 (55.2%)	3.7 (1.5 to 9.4)	
Asian	1 (1.3%)	0 (0.0%)	0.0 (0.0 to ∞)	
Other	2 (2.6%)	2 (6.9%)	4.9 (0.6 to 38.7)	
Parity				**0.03**
0	38 (48.7%)	9 (31%)	--- Reference ---	
1	31 (39.7%)	10 (34.5%)	1.4 (0.5 to 3.8)	
≥2	9 (11.5%)	10 (34.5%)	4.7 (1.5 to 14.9)	
Previous GDM history	39 (50.0%)	17 (58.6%)		
No	38 (48.7%)	38 (48.7%)		
Not applicable (para 0)	0 (0.0%)	2 (6.9%)		
Unknown	1 (1.3%)	1 (3.4%)		
Yes	1/40 (2.5%)	1/18 (5.6%)	2.2 (0.2 to 33.6)	0.56
History of GDM *(multiparous only)*				
Family history				
T1DM	2/78 (2.6%)	0/29 (0.0%)		
T2DM	16/78 (20.5%)	7/29 (24.1%)		
Smoking				0.33
Never	45 (57.7%)	21 (72.4%)	--- Reference ---	
Ex-smoker	28 (35.9%)	6 (20.7%)	0.5 (0.2 to 1.3)	
Current	5 (6.4%)	2 (6.9%)	0.9 (0.2 to 4.8)	

*Results are given as n (%) or mean (SD)

** Comparisons are differences in the mean, median differences or odds ratios as appropriate. Values are left blank where there are insufficient data. Comparisons are adjusted for baseline levels throughout.

The randomised treatment allocation is balanced by minimisation on maternal age, centre, ethnicity and parity

5.2 Maternal anthropometry and biochemistry by randomisation group

5.2.1 Anthropometry

No significant difference in skinfold thickness (triceps, biceps, subscapular and suprailiac) including sum of skinfolds was observed following the intervention (Table 17**Error! Reference source not found.**) and gestational weight gain in the two groups was equivalent ($Ch^2(1)=2.23$, p=0.33).

Table 17 Summaries of skinfold thickness (mm) by randomised treatment

	Intervention (n=57)	Control (n=58)	Treatment effect: Difference in arithmetic means (95% CI)	P*
Triceps				
Baseline	33.9 (8.4)[1]	32.4 (9.0)[4]	-	
Post-intervention	34.3 (7.3)[2]	32.4 (8.7)[5]	0.6 (-2.4 to 3.5)	0.7
Late gestation	33.1 (7.9)[3]	32.0 (7.0)[6]	-0.01(-3.5 to 3.4)	1.0
Biceps				
Baseline	26.0 (8.7)[1]	25.9 (8.5)[7]	-	-
Post-intervention	25.3 (7.7)[2]	25.3 (8.5)[5]	0.3 (-2.8 to 3.3)	0.9
Late gestation	24.2 (7.7)[3]	24.5 (6.8)[6]	-0.1(-3.5 to 3.3)	0.9
Subscapular				
Baseline	34.8 (9.1)[1]	33.3 (9.9)[4]	-	-
Post-intervention	36.6 (7.9)[2]	35.5 (10.0)[5]	0.3 (-2.9 to 3.6)	0.8
Late gestation	37.4 (8.5)[3]	36.5 (8.0)[6]	0.5 (-3.2 to 4.2)	0.8
Suprailiac				
Baseline	29.4 (9.3)[1]	31.2 (8.7)[4]	-	-
Post-intervention	32.5 (8.8)[2]	31.2 (7.9)[5]	4.1 (1.2 to 6.9)	0.006
Late gestation	32.8 (9.7)[3]	31.6 (9.0)[6]	4.2 (0.6 to 7.9)	0.02
Sum of skinfolds				
Baseline	90.1 (20.5)[1]	90.6 (18.7)[7]	-	-
Post-intervention	94.4 (18.6)[2]	92.0 (20.6)[5]	4.5 (-1.5 to 10.5)	0.1
Late gestation	94.4 (19.2)[3]	92.6 (18.4)[6]	4.4 (-2.8 to 11.6)	0.2

Data are presented as n=arithmetic mean (SD)

[1]n=59, [2]n=53, [3]n=46, [4]n=58, [5]n=54, [6]n=44, [7]n=57

Baseline (15^{+0}-17^{+6} weeks'), Post-intervention (27^{+0}-28^{+6}) weeks, late gestation (34^{+0}-35^{+6})

* Effect of randomised treatment estimated by random effects Generalised Least Squares (GLS) regression clustering by patient with robust standard errors and adjustment for visit and GDM status

Similarly there were no differences in circumference measurements (neck, waist, mid-arm, wrist, hip and thigh) see Appendix 2: UPBEAT pilot results, page 211.

5.2.2 Biochemistry

Following completion of the dietary and physical intervention at 27^{+0}-28^{+6} weeks' gestation, no differences were observed between the two arms. In late gestation however (34^{+0}-35^{+6} weeks'), the concentration of cholesterol, LDL and visfatin were lower in the intervention arm (Table 18). Treatment effects were estimated using multiple regression, with an adjustment for baseline measurements.

Table 18 Summaries of biomarker concentration by randomised treatment at three time points

Biomarker	Intervention (n=57)	Control (n=58)	Treatment effect: Ratio of geometric means (95% CI)	P*
Fructosamine (umol/l)				
Baseline	194.33 (1.10)[1]	195.22 (1.09)[9]	-	-
Post-intervention	187.28 (1.11)[2]	189.92 (1.10)[4]	0.99 (0.96 to 1.02)	0.58
Late gestation	181.26 (1.10)[3]	189.87 (1.09)[10]	0.98 (0.95 to 1.01)	0.15
ALT (U/L)				
Baseline	19.47 (1.64)[1]	19.35 (1.65)[9]	-	-
Post-intervention	17.91 (1.48)[2]	16.43 (1.46)[4]	1.07 (0.91 to 1.26)	0.42
Late gestation	16.77 (1.48)[3]	17.55 (1.53)[10]	0.97 (0.79 to 1.18)	0.74
AST (U/L)				
Baseline	26.41 (1.44)[1]	25.90 (1.47)[9]	-	-
Post-intervention	24.62 (1.35)[2]	21.67 (1.98)[2]	1.09 (0.91 to 1.32)	0.36
Late gestation	26.78 (1.35)[3]	25.73 (1.34)[10]	1.02 (0.90 to 1.17)	0.73
Ferritin (ng/ml)				
Baseline	46.55 (2.54)[1]	33.65 (1.94)[9]	-	-
Post-intervention	18.91 (2.39)[2]	13.11 (2.17)[4]	1.03 (0.79 to 1.33)	0.83
Late gestation	17.57 (1.93)[3]	13.16 (1.83)[5]	1.01 (0.76 to 1.35)	0.93
Adiponectin (µg/ml)				
Baseline	6.72 (1.89)[1]	6.38 (1.74)[9]	-	-
Post-intervention	5.90 (1.84)[2]	5.39 (2.08)[2]	1.07 (0.89 to 1.29)	0.44
Late gestation	6.22 (1.83)[3]	5.74 (1.67)[10]	1.05 (0.88 to 1.24)	0.60
tPA (ng/ml)				
Baseline	9.33 (1.46)[1]	9.08 (1.52)[9]	-	-
Post-intervention	11.24 (1.47)[2]	10.27 (1.62)[4]	1.05 (0.90 to 1.24)	0.53
Late gestation	12.06 (1.42)[3]	11.73 (1.49)[5]	1.04 (0.90 to 1.21)	0.57
IL-6 (pg/ml)				
Baseline	0.97 (2.67)[1]	1.01 (2.09)[2]	-	-
Post-intervention	1.12 (2.58)[4]	1.13 (2.35)[11]	1.06 (0.75 to 1.48)	0.75
Late gestation	1.19 (2.32)[5]	1.18 (2.01)[5]	1.15 (0.84 to 1.58)	0.39
Leptin (pg/ml)				
Baseline	60.27 (1.52)[6]	55.11 (1.49)[7]	-	-
Post-intervention	59.34 (1.86)[7]	60.37 (1.43)[7]	0.91 (0.76 to 1.09)	0.29
Late gestation	53.00 (1.77)[3]	50.35 (1.51)[12]	0.96 (0.82 to 1.13)	0.64
Visfatin (ng/ml)				
Baseline	5.54 (1.36)[6]	4.97 (1.47)[7]	-	-
Post-intervention	5.36 (1.40)[2]	5.06 (1.60)[7]	0.97 (0.86 to 1.08)	0.57
Late gestation	4.89 (1.38)[3]	5.15 (1.52)[10]	0.85 (0.75 to 0.97)	0.02
Insulin (mU/l)				
Baseline	24.63 (2.69)[1]	18.53 (2.90)[13]	-	-

Biomarker	Intervention (n=57)	Control (n=58)	Treatment effect: Ratio of geometric means (95% CI)	P*
Post-intervention	18.61 (2.39)[7]	14.62 (1.99)[2]	0.96 (0.60 to 1.53)	0.85
Late gestation	33.51 (2.90)[8]	31.87 (2.75)[10]	0.80 (0.47 to 1.35)	0.40
Cholesterol (mmol/l)				
Baseline	5.28 (1.21)[1]	5.48 (1.18)[13]	-	-
Post-intervention	5.79 (1.25)[7]	6.07 (1.21)[7]	0.98 (0.94 to 1.02)	0.37
Late gestation	5.93 (1.28)[8]	6.63 (1.21)[10]	0.94 (0.89 to 0.99)	0.03
Triglycerides (mmol/l)				
Baseline	1.54 (1.38)[1]	1.61 (1.37)[13]	-	-
Post-intervention	1.91 (1.41)[7]	1.92 (1.48)[13]	1.03 (0.94 to 1.14)	0.48
Late gestation	2.50 (1.49)[8]	2.52 (1.42)[10]	1.02 (0.91 to 1.13)	0.78
HDL (mmol/l)				
Baseline	1.72 (1.27)[1]	1.63 (1.27)[13]	-	-
Post-intervention	1.75 (1.28)[7]	1.73 (1.33)[7]	0.95 (0.90 to 1.01)	0.09
Late gestation	1.69 (1.35)[8]	1.72 (1.30)[10]	0.93 (0.84 to 1.03)	0.15
CRP (mg/l)				
Baseline	7.75 (2.45)[1]	9.18 (1.91)[13]	-	-
Post-intervention	5.83 (2.46)[7]	8.20 (1.87)[7]	0.84 (0.67 to 1.05)	0.13
Late gestation	5.53 (2.33)[8]	5.19 (1.94)[10]	1.26 (0.97 to 1.65)	0.08
VLDL (mmol/l)				
Baseline	0.70 (1.38)[1]	0.74 (1.37)[13]	-	-
Post-intervention	0.87 (1.41)[7]	0.88 (1.48)[7]	1.05 (0.95 to 1.15)	0.35
Late gestation	1.14 (1.49)[8]	1.15 (1.42)[10]	1.03 (0.92 to 1.14)	0.65
LDL (mmol/l)				
Baseline	2.77 (1.36)[1]	3.02 (1.33)[13]	-	-
Post-intervention	2.95 (1.47)[7]	3.21 (1.38)[7]	0.99 (0.89 to 1.09)	0.82
Late gestation	2.90 (1.58)[8]	3.65 (1.33)[10]	0.89 (0.81 to 0.98)	0.02
Cholesterol:HDL				
Baseline	3.07 (1.26)[1]	3.37 (1.29)[13]	-	-
Post-intervention	3.32 (1.34)[7]	3.52 (1.33)[7]	1.03 (0.98 to 1.09)	0.27
Late gestation	3.51 (1.42)[8]	3.86 (1.34)[10]	1.01 (0.91 to 1.12)	0.81
LDL:HDL				
Baseline	1.61 (1.47)[1]	1.86 (1.47)[13]	-	-
Post-intervention	1.69 (1.61)[7]	1.86 (1.51)[7]	1.04 (0.93 to 1.17)	0.52
Late gestation	1.71 (1.70)[8]	2.13 (1.48)[10]	0.96 (0.84 to 1.10)	0.55

Data are presented as geometric mean (geometric SD; defined as exponent of SD of logged value)

Baseline (15^{+0}-17^{+6} weeks'), Post-intervention (27^{+0}-28^{+6} weeks'), Late gestation (34^{+0}-35^{+6} weeks')

[1]n=59. [2]n=52, [3]n=43, [4]n=51, [5]n=40, [6]n=57. [7]n=53, [8]n=45, [9]n=55, [10]n=41, [11]n=49, [12]n=42, [13]n=56

* Effect of randomised treatment estimated by random effects Generalised Least Squares (GLS) regression clustering by patient with robust standard errors and adjustment for visit and GDM status. Comparisons are adjusted for baseline levels throughout.

1. Mean plasma cholesterol concentration was significantly lower in the intervention group in late pregnancy (34^{+0}-35^{+6} weeks') (5.93mmol/l v 6.63mmol/l, ratio 0.94 [95% CI 0.89 to 0.99] or -6% change [CI -11 to -1%], p=0.03) (Figure 17).

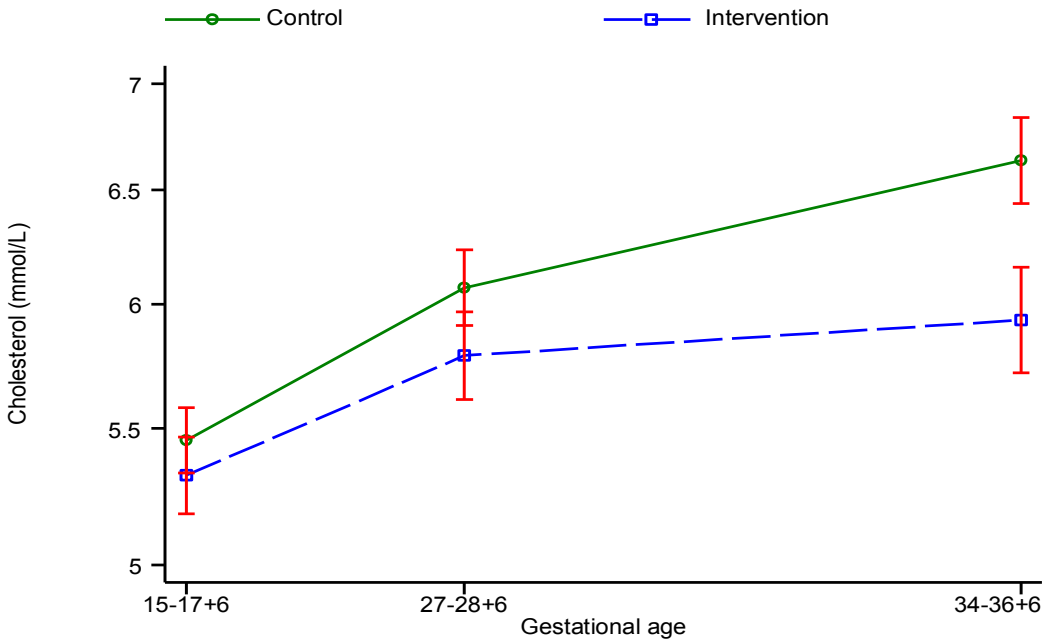
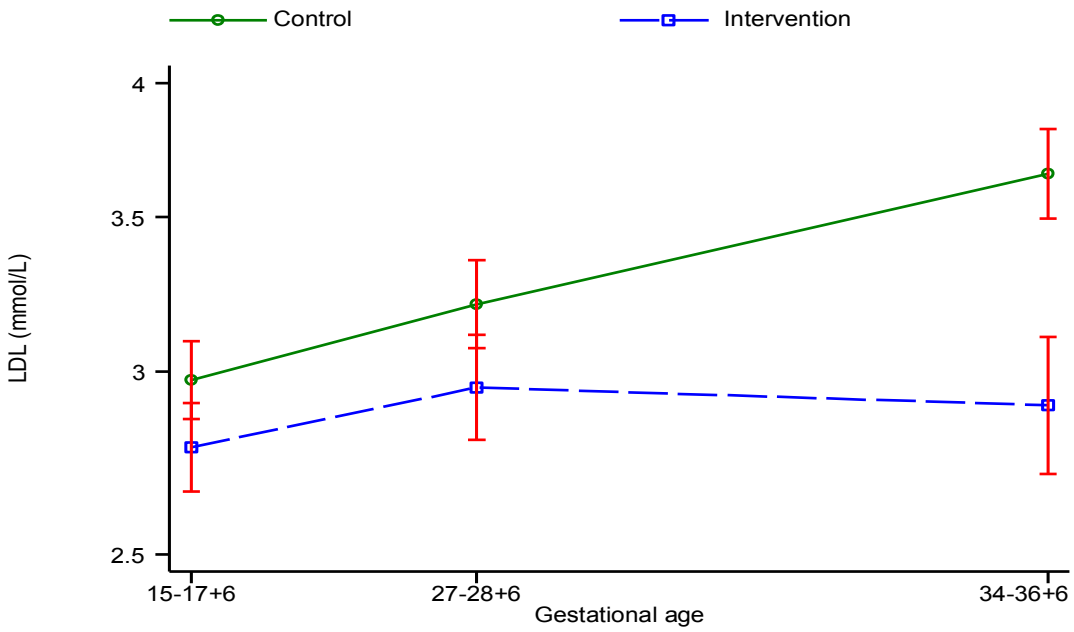


Figure 17 Longitudinal changes in plasma cholesterol concentration by randomisation. Error bars represent geometric mean±SEM on the log scale.

2. Mean plasma LDL concentration was 2.90mmol/l v 3.65mmol/l, for intervention and control respectively at visit 5 (ratio 0.89 [95% CI 0.81 to 0.98], p=0.02), representing an 11% reduction (-11% change, [95% CI -18.9 to -2.1%]). LDL progressively increased in the control group (Figure 18).

   a)



b)

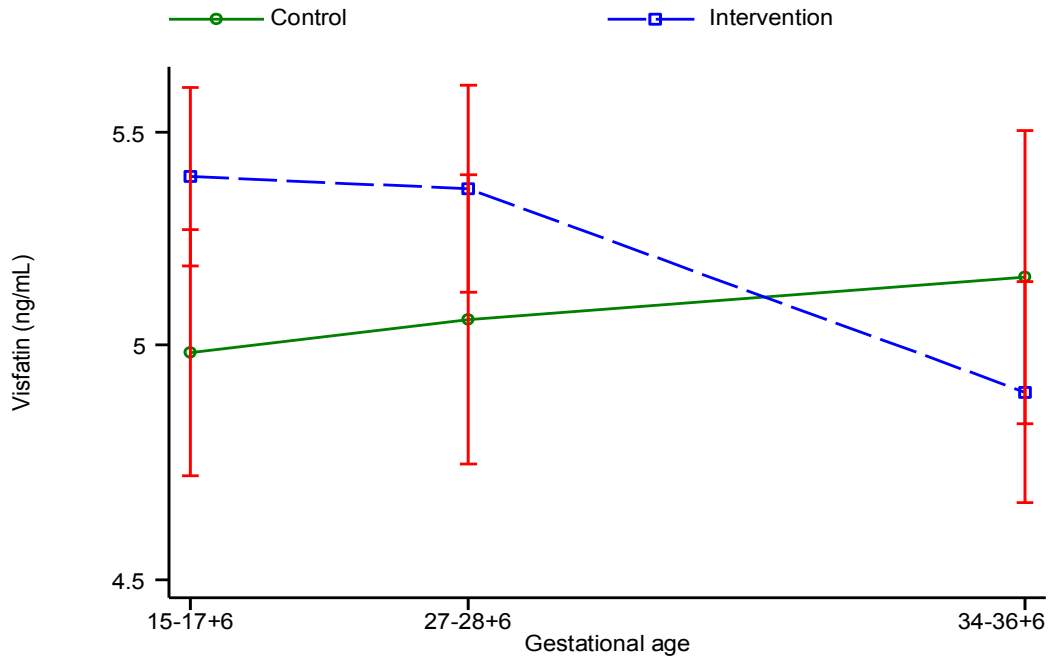


Figure 18a) Longitudinal changes in plasma LDL concentration and in plasma visfatin (b) concentration by randomisation. Error bars represent geometric mean±SEM on the log scale.

3. Plasma visfatin concentrations were highly variable. Values were higher at recruitment in the intervention arm and remained stable until completion of the programme (27^{+0}-28^{+6} weeks' gestation) when a progressive decline was observed. This is in contrast to the control arm, which demonstrated a linear increase (Figure 18).

5.3 Maternal anthropometry and biochemistry by GDM status

Data for 11 women was excluded from all analysis of the biomarkers by GDM status due to incomplete OGTT results. Of the 107 women included, 29 (27.4%) developed GDM defined by the IADPSG criteria. For the 16 biomarkers measured, including two clinically relevant ratios (cholesterol: HDL and LDL: HDL), comparisons were made for each study visit between women with and without GDM following logarithmic transformation of each variable.

5.3.1 Anthropometry

At baseline (15^{+0}-17^{+6} weeks'), women who developed GDM had significantly greater triceps skinfolds (p=0.003) and total sum of skinfold measures (37.4 v 31.2mm, p=0.03). Following analysis of the data for all visits combined, only triceps remained significant (35.1 v 32.0mm, p=0.045) (**Error! Reference source not found.**).

Table 19 Summaries of skinfold thickness (mm) by GDM status

	GDM (n=29)	No GDM (n=78)	Overall comparison between groups: Mean difference (95% CI), Significance test*	P
Triceps			3.1 (0.1 to 6.1), 0.045	
Baseline	37.4 (10.2)[1]	31.2 (7.4)[3]		
Post-intervention	36.0 (8.2)[1]	32.4(7.8)[3]		
Late gestation	32.0 (7.8)[2]	32.8 (7.4)[4]		
Biceps			1.7 (-1.2 to 4.7), 0.25	
Baseline	28.0 (9.5)[1]	24.4 (7.5)[5]		
Post-intervention	27.1 (8.5)[1]	24.6 (7.9)[3]		
Late gestation	23.7 (7.4)[2]	24.6 (7.2)[4]		
Subscapular			4.2 (1.6 to 6.8), 0.002	
Baseline	36.0 (8.2)[1]	32.2 (9.1)[3]		
Post-intervention	40.1 (6.7)[1]	34.6 (9.3)[3]		
Late gestation	38.9 (7.3)[2]	36.1 (8.5)[4]		
Suprailiac			-0.99 (-4.0 to 2.0), 0.52	
Baseline	29.9 (8.3)[1]	29.6 (8.3)[3]		
Post-intervention	31.4 (7.8)[1]	32.0 (8.6)[3]		
Late gestation	29.9 (8.6)[2]	33.2 (9.5)[4]		
Sum of skinfolds			5.0 (-1.4 to 11.4), 0.13	
Baseline	93.9 (16.5)[1]	86.0 (16.7)[5]		
Post-intervention	98.6 (16. 7)[1]	91.2 (20.2)[3]		
Late gestation	92.5 (19.9)[2]	93.9 (18.4)[4]		

Data are presented as n=arithmetic mean (SD)

[1]n=29, [2]n=26, [3]n=78, [4]n=64, [5]n=77

Visit 2: randomisation (16^{+0}-18^{+6}), visit 3: post intervention (27^{+0}-28^{+6}), visit 5: late gestation (34^{+0}-35^{+6})

*Comparison between GDM & non-GDM women by random effects Generalised Least Squares (GLS) regression clustering by patient with robust standard errors and adjustment for visit and randomised intervention (Arellano 1987).

No differences were observed between circumference measures see Appendix 2: UPBEAT pilot results, page 211.

5.3.2 Biochemistry

Details of the mean concentration at each study visit for individual biomarkers are shown in **Table 20**.

Table 20 Summaries of biomarker concentration by GDM status

Biomarker	GDM (n=29)	No GDM (n=78)	Comparison between groups: Ratio of geometric means (95% CI)	Overall comparison between groups: Ratio of geometric means (95% CI), Significance test
Fructosamine (umol/l)				1.06 (1.02 to 1.10)
Baseline	200.87 (1.10)[1]	192.90 (1.09)[7]	1.04 (1.00 to 1.09)	0.003
Post-intervention	198.44 (1.11)[2]	184.85 (1.10)[8]	1.08 (1.03 to 1.12)	
Late gestation	191.85 (1.10)[3]	182.75 (1.09)[9]	1.05 (1.01 to 1.10)	
ALT (U/L)				1.06 (0.90 to 1.24)
Baseline	21.41 (1.79)[1]	19.00 (1.57)[7]	1.13 (0.89 to 1.44)	0.48
Post-intervention	16.84 (1.47)[2]	17.29 (1.47)[8]	0.98 (0.83 to 1.15)	
Late gestation	17.49 (1.59)[3]	17.00 (1.47)[9]	1.08 (0.88 to 1.33)	
AST (U/L)				1.18 (1.04 to 1.34)
Baseline	30.63 (1.53)[1]	25.07 (1.41)[7]	1.23 (1.03 to 1.46)	0.01
Post-intervention	25.67 (1.36)[2]	22.18 (1.80)[10]	1.16 (0.97 to 1.38)	
Late gestation	28.68 (1.38)[3]	25.30 (1.32)[9]	1.15 (1.00 to 1.33)	
Ferritin (ng/ml)				1.13 (0.86 to 1.48)
Baseline	42.06 (2.27)[1]	39.48 (2.29)[7]	1.05 (0.74 to 1.49)	0.40
Post-intervention	17.71 (2.10)[2]	15.07 (2.40)[8]	1.18 (0.85 to 1.63)	
Late gestation	15.93 (1.89)[3]	15.01 (1.92)[11]	1.16 (0.88 to 1.54)	
Adiponectin (µg/ml)				0.73 (0.59 to 0.91)
Baseline	4.97 (1.72)[1]	7.34 (1.76)[7]	0.66 (0.52 to 0.84)	0.005
Post-intervention	4.56 (1.73)[2]	6.12 (2.01)[10]	0.74 (0.58 to 0.95)	
Late gestation	5.00 (1.57)[3]	6.45 (1.80)[9]	0.83 (0.66 to 1.04)	
tPA (ng/ml)				1.12 (0.97 to 1.30)
Baseline	10.35 (1.49)[1]	9.00 (1.47)[7]	1.14 (0.96 to 1.35)	0.13
Post-intervention	12.40 (1.54)[2]	10.16 (1.53)[8]	1.22 (1.02 to 1.47)	
Late gestation	11.59 (1.47)[3]	12.04 (1.44)[11]	0.98 (0.83 to 1.17)	
IL-6 (pg/ml)				0.98 (0.73 to 1.31)
Baseline	1.01 (2.08)[4]	0.95 (2.54)[10]	1.03 (0.73 to 1.46)	0.89
Post-intervention	1.11 (2.29)[1]	1.14 (2.54)[12]	1.02 (0.70 to 1.48)	
Late gestation	1.05 (1.79)[5]	1.25 (2.31)[13]	0.88 (0.64 to 1.21)	
Leptin (pg/ml)				0.84 (0.71 to 0.99)
Baseline	53.82 (1.49)[1]	59.36 (1.52)[8]	0.91 (0.76 to 1.07)	0.04
Post-intervention	55.94 (1.51)[2]	61.39 (1.70)[7]	0.91 (0.75 to 1.10)	
Late gestation	40.46 (1.66)[3]	57.21 (1.59)[14]	0.69 (0.56 to 0.86)	
Visfatin (ng/ml)				0.96 (0.84 to 1.09)
Baseline	4.94 (1.40)[1]	5.28 (1.42)[8]	0.93 (0.80 to 1.07)	0.53
Post-intervention	5.12 (1.36)[2]	5.24 (1.56)[15]	0.97 (0.84 to 1.13)	
Late gestation	4.82 (1.31)[3]	5.11 (1.51)[9]	0.98 (0.85 to 1.14)	
Insulin (mU/l)				1.32 (1.01 to 1.73)
Baseline	25.75 (2.94)[2]	20.20 (2.78)[7]	1.28 (0.80 to 2.04)	0.04
Post-intervention	17.74 (1.94)[2]	16.07 (2.31)[15]	1.10 (0.82 to 1.49)	
Late gestation	46.68 (2.87)[6]	28.04 (2.70)[14]	1.71 (1.05 to 2.79)	
Cholesterol (mmol/l)				0.94 (0.87 to 1.01)
Baseline	5.31 (1.18)[2]	5.42 (1.21)[7]	0.98 (0.91 to 1.05)	0.10
Post-intervention	5.62 (1.21)[2]	6.04 (1.24)[7]	0.93 (0.86 to 1.01)	
Late gestation	5.78 (1.24)[6]	6.48 (1.25)[14]	0.89 (0.81 to 0.98)	
Triglycerides (mmol/l)				1.05 (0.91 to 1.21)
Baseline	1.67 (1.42)[2]	1.53 (1.38)[7]	1.09 (0.94 to 1.26)	0.51

Biomarker	GDM (n=29)	No GDM (n=78)	Comparison between groups: Ratio of geometric means (95% CI)	Overall comparison between groups: Ratio of geometric means (95% CI), Significance test
Post-intervention	2.05 (1.48)[2]	1.87 (1.42)[7]	1.10 (0.93 to 1.29)	
Late gestation	2.39 (1.44)[6]	2.56 (1.46)[14]	0.96 (0.81 to 1.13)	
HDL (mmol/l)				0.96 (0.86 to 1.07)
Baseline	1.64 (1.32)[2]	1.71 (1.26)[7]	0.96 (0.86 to 1.07)	0.44
Post-intervention	1.66 (1.39)[2]	1.77 (1.27)[7]	0.94 (0.82 to 1.07)	
Late gestation	1.72 (1.34)[6]	1.70 (1.32)[14]	0.98 (0.85 to 1.12)	
CRP (mg/l)				1.06 (0.81 to 1.38)
Baseline	9.18 (1.93)[2]	7.77 (2.30)[7]	1.19 (0.88 to 1.63)	0.66
Post-intervention	7.37 (1.75)[2]	6.75 (2.37)[7]	1.11 (0.84 to 1.47)	
Late gestation	4.96 (2.08)[6]	5.55 (2.18)[14]	0.87 (0.62 to 1.21)	
VLDL (mmol/l)				1.05 (0.91 to 1.21)
Baseline	0.76 (1.42)[2]	0.71 (1.38)[7]	1.08 (0.93 to 1.25)	0.52
Post-intervention	0.94 (1.48)[2]	0.86 (1.42)[7]	1.10 (0.93 to 1.29)	
Late gestation	1.09 (1.44)[6]	1.17 (1.46)[14]	0.96 (0.81 to 1.13)	
LDL (mmol/l)				0.89 (0.78 to 1.02)
Baseline	2.74 (1.39)[2]	2.93 (1.34)[7]	0.93 (0.82 to 1.07)	0.09
Post-intervention	2.84 (1.43)[2]	3.17 (1.42)[7]	0.90 (0.77 to 1.04)	
Late gestation	2.84 (1.45)[6]	3.43 (1.50)[14]	0.84 (0.72 to 0.98)	
Cholesterol:HDL				0.98 (0.88 to 1.09)
Baseline	3.23 (1.31)[2]	3.17 (1.27)[7]	1.02 (0.92 to 1.13)	0.70
Post-intervention	3.39 (1.37)[2]	3.42 (1.32)[7]	0.99 (0.87 to 1.13)	
Late gestation	3.36 (1.36)[6]	3.81 (1.39)[14]	0.91 (0.79 to 1.05)	
LDL:HDL				0.93 (0.78 to 1.10)
Baseline	1.67 (1.56)[2]	1.71 (1.45)[7]	0.97 (0.82 to 1.16)	0.41
Post-intervention	1.71 (1.61)[2]	1.80 (1.55)[7]	0.96 (0.79 to 1.16)	
Late gestation	1.65 (1.61)[6]	2.02 (1.60)[14]	0.86 (0.70 to 1.05)	

Results given as geometric means (SD)

Baseline (15^{+0}-17^{+6} weeks'), Post-intervention (27^{+0}-28^{+6} weeks'), Late gestation (34^{+0}-35^{+6} weeks')

[1]n=28, [2]n=29, [3]n=25, [4]n=27, [5]n=24, [6]n=26, [7]n=77, [8]n=74, [9]n=59, [10]n=75, [11]n=58, [12]n=72, [13]n=56, [14]n=60, [15]n=76

*Mean difference across all visits.

**Comparison between GDM & non-GDM women by random effects Generalised Least Squares (GLS) regression clustering by patient with robust standard errors and adjustment for visit and randomised intervention.

1. Concentrations of plasma fructosamine, were significantly greater in women who developed GDM, (6% difference; 95% CI 1.9 to 9.6%) over the duration of the study (ratio 1.06 [95% CI 1.02 to 1.10], p=0.003). There was a steady decline in fructosamine concentrations from early pregnancy in both GDM and non GDM (Figure 19).

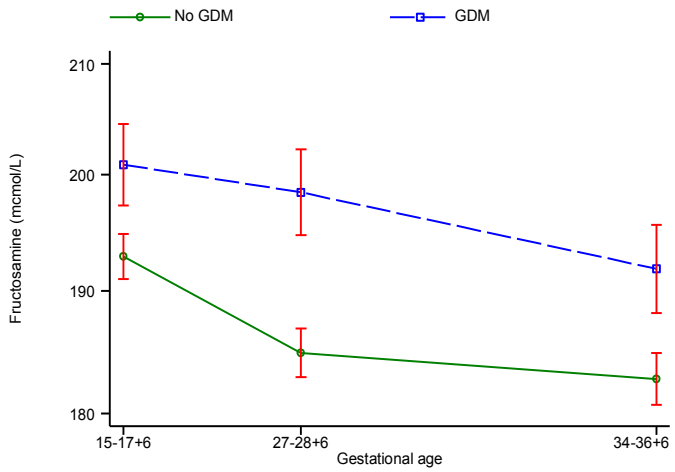


Figure 19 Longitudinal changes in plasma fructosamine concentration by GDM status. Error bars represent geometric mean±SEM on the log scale.

2. Plasma concentrations of AST were significantly greater in women with GDM representing an overall 18% increase (95% CI 3.9 to 34.1) across the study (ratio 1.18, [95% CI 1.04 to 1.43], p=0.01).

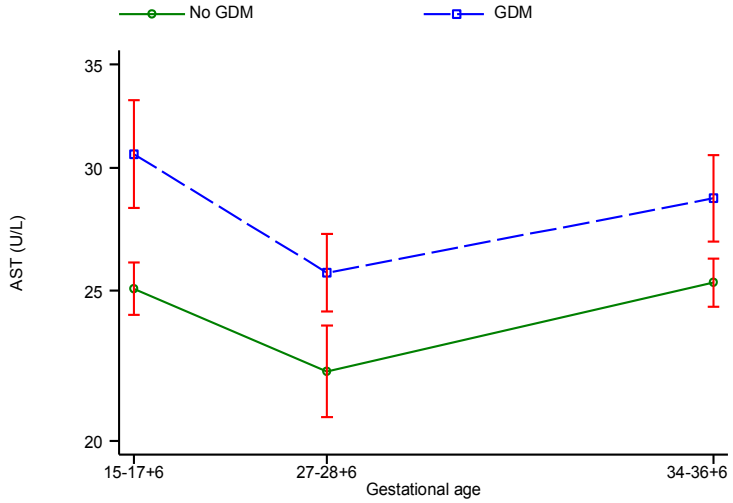


Figure 20 Longitudinal changes in plasma AST concentration by GDM status. Error bars represent geometric mean±SEM on the log scale.

3. In both groups of women, adiponectin concentrations declined from visits 2 to 3, followed by a small rise in late gestation, without a return to baseline (**Figure 21**). Women with GDM had lower concentrations of adiponectin throughout the study. At randomisation, a -34% difference was observed for women who subsequently went on to develop GDM (ratio 0.66 [95%CI 0.52

to 0.84], p=0.001), which reduced to -17% by visit 3 (ratio 0.83, [95%CI 0.66 to 1.04]). The difference across all visits was -27% (95%CI 9.1 to 40.7%) (**Table 20**).

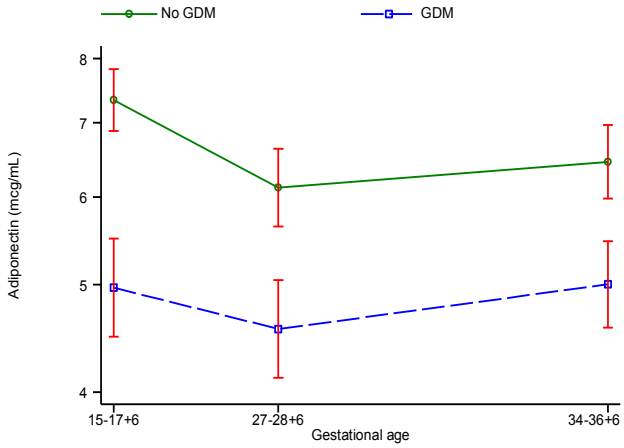


Figure 21 Longitudinal changes in plasma adiponectin concentration by GDM status. Error bars represent geometric mean±SEM on the log scale.

4. Women who developed GDM tended to have lower concentrations of plasma leptin throughout pregnancy. At late gestation, a significant 31% difference between those with and without GDM was observed (ratio 0.69 [95%CI 0.85 to 1.14], p=0.001) (**Figure 22**). This difference resulted mainly from a reduction in leptin levels in the GDM group, while the levels in the controls remained relatively stable.

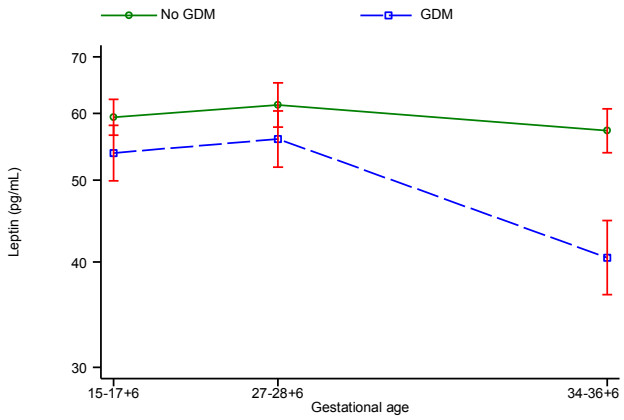


Figure 22 Longitudinal changes in plasma leptin concentration by GDM status. Error bars represent geometric mean±SEM on the log scale.

5. Plasma insulin concentrations decreased from randomisation to post intervention in both groups and then increased thereafter. Insulin concentrations were similar at these time periods whereas in late gestation, concentrations were significantly greater in women with GDM compared to those without (ratio 1.71 [95%CI 1.05 to 2.79], p=0.03 at visit 3) (**Figure 23**).

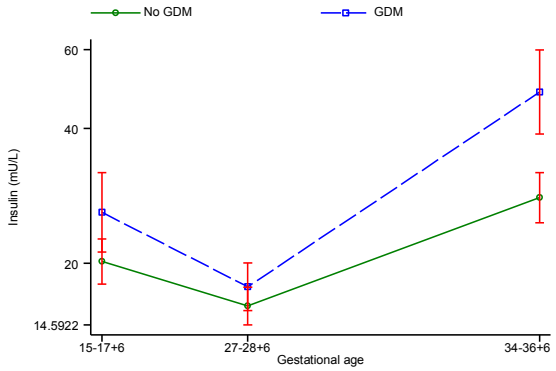


Figure 23 Longitudinal changes in plasma insulin concentration by GDM status. Error bars represent geometric mean±SEM on the log scale.

5.4 NEFA and fatty acids

Total fasting plasma NEFA and fatty acid composition were measured on post intervention samples only obtained during the OGTT at visit 3 (27^{+0}-28^{+6} weeks' gestation).

5.4.1 Comparison of NEFA and fatty acid composition by randomisation

Since abnormal fatty acids (FAs) are associated with insulin resistance and fetal macrosomia and because significant differences were found in the dietary intake of saturated fatty acids and the ratio of PUFA:SFA, a detailed analysis of fatty acids was undertaken (Poston, Briley et al. 2013).

A significant 13% reduction was observed for the intervention arm compared to the control in the concentration of the SFA myristic acid (C14:0) (p=0.027).

Greater concentrations of the SFA stearic acid (C18:0) (p=0.006) and a borderline significance reduction of MUFA oleic acid (C18:3n-6) (p=0.06) were found in the intervention group.

All other plasma FAs were equivalent between the randomised groups (Table 20).

Table 21 Summary of NEFA concentration and composition of fatty acids by randomisation at 27^{+0}-28^{+6} week's gestation

NEFA and fatty acid composition (mmol/L)	Control (n=52)	Intervention (n=52)	Comparison: difference in arithmetic means (95% CI)*	P
NEFA (mmol/L)	0.31 (0.17)	0.33 (0.17)	0.02 (-0.05 to 0.09)	0.56
Plasma 14:0	0.92 (0.35)	0.79 (0.25)	-0.13 (-0.25 to -0.02)	0.027
Plasma 16:0	23.18 (2.00)	22.92 (1.85)	-0.25 (-1.00 to 0.50)	0.50
Plasma 16:1	1.89 (0.76)	1.69 (0.71)	-0.21 (-0.49 to 0.08)	0.15
Plasma 18:0	5.29 (0.46)	5.56 (0.52)	0.27 (0.08 to 0.46)	0.006
Plasma 18:1n-9	22.65 (2.84)	22.47 (2.69)	-0.18 (-1.25 to 0.90)	0.74
Plasma 18:1n-7	1.62 (0.27)	1.61 (0.22)	-0.01 (-0.11 to 0.08)	0.78
Plasma 18:2n-6	25.98 (3.81)	26.28 (3.51)	0.30 (-1.12 to 1.73)	0.68
Plasma 18:3n-3	0.74 (0.20)	0.68 (0.22)	-0.05 (-0.13 to 0.03)	0.20
Plasma 18:3n-6	0.25 (0.09)	0.22 (0.07)	-0.03 (-0.06 to 0.00)	0.06
Plasma 20:3n-6	1.80 (0.41)	1.69 (0.37)	-0.12 (-0.27 to 0.04)	0.14
Plasma 20:4n-6	6.42 (1.25)	6.85 (1.26)	0.43 (-0.06 to 0.91)	0.09
Plasma 20:5n-3 *	0.66 (0.84)	0.66 (0.54)	-0.00 (-0.27 to 0.27)	0.996
Plasma 22:4n-6	0.24 (0.07)	0.23 (0.06)	-0.01 (-0.03 to 0.02)	0.50
Plasma 22:5n-6	0.63 (0.20)	0.63 (0.17)	0.01 (-0.07 to 0.08)	0.86
Plasma 22:5n-3	0.36 (0.08)	0.37 (0.12)	0.02 (-0.02 to 0.05)	0.43
Plasma 22:6n-3	2.82 (0.78)	2.95 (0.79)	0.13 (-0.18 to 0.43)	0.42
Other plasma fatty acids	4.55 (0.62)	4.40 (0.60)	-0.15 (-0.39 to 0.09)	0.21

Results given as arithmetic mean (SD). Comparison between control and intervention was by simple linear regression with robust standard errors.

* For plasma 20:5n-3, results are confirmed by a non-parametric Wilcoxon rank-sum test

5.4.2 Comparison of NEFA and fatty acid composition by GDM status

Plasma NEFA was significantly greater in subjects with GDM (p=0.037) and although not significant, concentrations of the PUFA eicosapentaenoic acid (EPA, plasma 20:5n-3), an omega-3, were 25% higher in GDM (p=0.28).

Women without GDM had greater concentrations of the naturally occurring trans vaccenic acid from the omega 7 group (C18:1n-7) (p=0.01) and dihomo-γ-linolenic acid (DGLA, C20:3n-6) (p=0.016) (**Table 22**).

Table 22 Summary of NEFA concentration and composition of fatty acids by GDM status at 27^{+0}-28^{+6} weeks' gestation

NEFA and fatty acid composition (mmol/L)	No GDM (n=75)	GDM (n=29)	Comparison: difference in arithmetic means (95% CI)*	P
NEFA	0.30 (0.16)	0.38 (0.19)	0.08 (0.01 to 0.16)	0.037
Plasma 14:0	0.85 (0.26)	0.87 (0.41)	0.02 (-0.14 to 0.19)	0.77
Plasma 16:0	22.81 (1.69)	23.66 (2.34)	0.85 (-0.09 to 1.79)	0.08
Plasma 16:1	1.82 (0.66)	1.72 (0.92)	-0.10 (-0.47 to 0.27)	0.593
Plasma 18:0	5.40 (0.46)	5.50 (0.62)	0.09 (-0.16 to 0.34)	0.46
Plasma 18:1n-9	22.59 (2.75)	22.49 (2.79)	-0.10 (-1.30 to 1.10)	0.87
Plasma 18:1n-7	1.65 (0.25)	1.52 (0.21)	-0.13 (-0.23 to -0.03)	0.01
Plasma 18:2n-6	26.22 (3.23)	25.89 (4.62)	-0.33 (-2.18 to 1.51)	0.72
Plasma 18:3n-3	0.73 (0.20)	0.65 (0.23)	-0.09 (-0.18 to 0.01)	0.07
Plasma 18:3n-6	0.23 (0.08)	0.23 (0.07)	-0.01 (-0.04 to 0.02)	0.64
Plasma 20:3n-6	1.80 (0.41)	1.61 (0.33)	-0.19 (-0.34 to -0.04)	0.016
Plasma 20:4n-6	6.66 (1.33)	6.56 (1.11)	-0.10 (-0.61 to 0.41)	0.69
Plasma 20:5n-3	0.62 (0.73)	0.77 (0.61)	0.15 (-0.13 to 0.43)	0.28
Plasma	0.24 (0.07)	0.23 (0.06)	-0.00 (-0.03 to 0.02)	0.94

22:4n-6				
Plasma 22:5n-6	0.63 (0.19)	0.63 (0.17)	-0.00 (-0.08 to 0.07)	0.95
Plasma 22:5n-3	0.36 (0.11)	0.37 (0.08)	0.01 (-0.03 to 0.04)	0.77
Plasma 22:6n-3	2.84 (0.77)	3.00 (0.81)	0.16 (-0.18 to 0.51)	0.36
Other plasma fatty acids	4.54 (0.56)	4.30 (0.71)	-0.24 (-0.53 to 0.05)	0.10

Results given as arithmetic mean (SD). Comparison between GDM and no GDM was by simple linear regression with robust standard errors.

* For plasma 20:5n-3, results are confirmed by a non-parametric Wilcoxon rank-sum test.

5.5 Prediction model for GDM

5.5.1 Biomarker analysis

Univariate biomarker analysis and development of the initial prediction model, included adjustment for maternal anthropometry (triceps skinfold and total sum of skinfolds) in addition to age, parity ≥2, black ethnicity, DBP and SBP as significant clinical predictors of GDM.

Plasma concentrations of AST at randomisation were significantly greater in women with GDM (ratio 1.23, 95% CI [10.01 to 1.50], p=0.042). Using clinically relevant categories of AST, (<20, 20-29.9 and ≥30U/L), the incidence of having GDM increased from 15.5% to 24.4% and 36.8% respectively. Odds ratios for development of GDM are shown in Table 23. These categories were not used in analysis for the prediction model however and as such the data was treated as continuous.

Table 23 Association of GDM with early pregnancy concentrations of plasma AST (16^{+0}-18^{+6} weeks' gestation)

AST (U/L)	**GDM (n=28/105)**	**No GDM (n=77/105)**	**Comparison (adjusted odds ratio, 95% CI)**
<20	4/26 (15.4%)	22/26 (84.6%)	[Reference group]
20-29.9	10/41 (24.4%)	31/41 (75.6%)	2.53 (0.43 to 17.79)
≥30	14/38 (36.8%)	24/38 (63.2%)	4.01 (0.71 to 22.52)

Results are given as n (row %). Comparisons are adjusted for triceps skinfold, total sum of skinfolds, age, parity ≥2, black ethnicity, DBP and SBP.

Plasma concentrations of adiponectin were 32% lower at baseline in subjects who subsequently developed GDM (ratio 0.68, 95% CI [0.54 to 0.85], p=0.001). When divided into 5 categories (<5, 5-9.9, 10-14.9, 15-19.9 and ≥20µg/ml), the risk of GDM was inversely associated with increasing concentrations of adiponectin. For concentrations ≥20µg/ml, there were no women with GDM but 3 women without (Table 24).

Table 24 Association of GDM with early pregnancy concentrations of adiponectin (16^{+0}-18^{+6} weeks' gestation).

Adiponectin (ug/ml)	GDM (n=28/105)	No GDM (n=77/105)	Comparison (adjusted odds ratio, 95%CI)
<5	12/32 (37.5%)	20/32 (62.5%)	[Reference group]
5-9.9	13/47 (27.7%)	34/47 (72.3%)	0.39 (0.11 to 1.44)
10-14.9	2/12 (16.7%)	12/12 (83.3%)	0.12 (0.01 to 1.92)
15-19.9	1/9 (11.1%)	8/9 (88.9%)	0.04 (0.00 to 1.67)
≥20	0/3 (0%)	3/3 (100%)	0

Results are given as n (row %) and adjusted for triceps skinfold, total sum of skinfolds, age, parity ≥2, black ethnicity, DBP and SBP

The distribution of AST and adiponectin by GDM status are illustrated below in a boxplot.

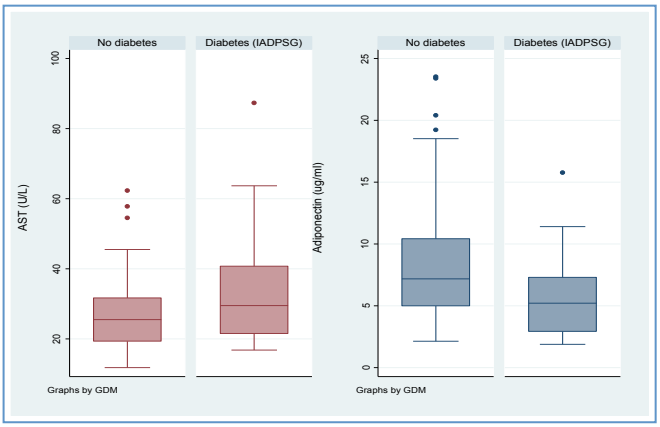


Figure 24 Box and whisker plot of the distribution of AST and adiponectin concentrations by GDM status. Boxes show median and quartiles; whiskers extend to the lowest value within 1.5 times the inter-quartile range (IQR) of the lower quartile, and the highest value within 1.5 IQR of the upper quartile (standard Tukey boxplots)**.**

In logistic regression, adiponectin (OR 0.18, 95%CI [0.05 to 0.67], p=0.01) and maternal age (OR 1.15, 95%CI [1.0 to 1.3], p=0.035) were consistently predictive of GDM. Black ethnicity (BE) ceased to be predictive (p=0.74) (Table 25).

Table 25 Combined logistic regression using biomarkers and all significant clinical risk factors (triceps and total sum of skinfolds, age, parity [≥2], Black ethnicity, SBP, DBP and adiponectin)

	Odds Ratio	95% Confidence Interval	P
AST	4.53	0.93 to 22.1	0.06
Log adiponectin	0.18	0.05 to 0.67	0.01
Age (for each additional year)	1.15	1.0 to 1.31	0.035
Parity ≥2	3.38	0.75 to 15.23	0.11
Black ethnicity	0.80	0.21 to 3.04	0.74
SBP	1.0	0.91 to 1.10	0.93
DBP	1.09	0.98 to 1.21	0.10
Triceps skinfolds	1.07	0.98 to 1.17	0.11
Total sum of skinfolds	1.0	0.96 to 1.05	0.82

Further analysis to explore why BE was no longer significant in the model found that BE was predictive of a high AST and low adiponectin in this cohort (p<0.001), which are both stronger predictors of GDM (Table 26).

Table 26 Comparison of early pregnancy concentrations of AST and adiponectin (16^{+0}-18^{+6} weeks' gestation) in Black and non-black subjects

	Black (n=36)	Non-black (n=69)	Comparison between groups: Ratio of geometric means (95% CI)	P
AST (U/L)	32.5 (1.4)	23.8 (1.4)	1.38 (1.20 to 1.57)	0.00
Adiponectin (ug/ml)	4.8 (1.8)	7.8 (1.7)	0.61 (0.49 to 0.77)	0.00

Data are presented as geometric mean (geometric SD; defined as exponent of SD of logged value)

5.5.1.1 Area under the curve-receiver operating curve (AUC-ROC)

The prediction model demonstrated an AUC-ROC of 0.796 (95% CI [0.692 to 0.898], p=0.073) for clinical predictors alone which increased to 0.857 (95% CI [0.777 to 0.938]) with the addition of adiponectin. The addition of AST resulted in a small, non-significant increase to 0.866 (95% CI 0.785 to 0.945, p=0.534).

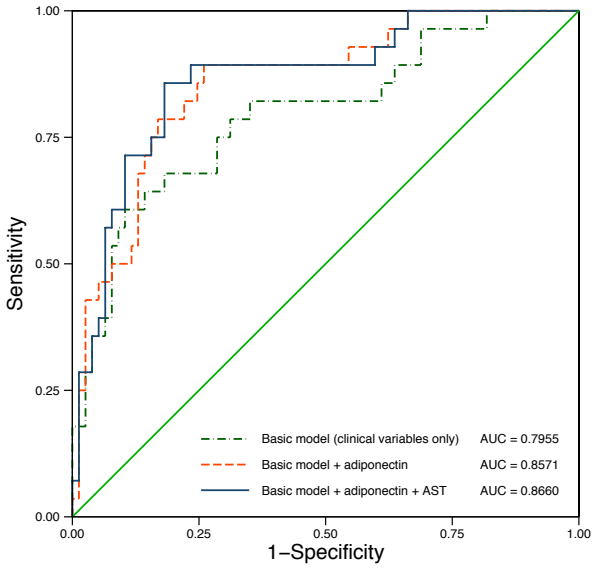


Figure 25 Receiver-operating curve and summaries using the basic model (including age, parity, ethnicity, blood pressure and maternal anthropometry), with the addition of adiponectin and AST. AUC, area under the receiver-operating curve.

5.5.2 Biomarker analysis excluding anthropometry

The role of maternal anthropometry in standard antenatal care has not been established. Therefore a decision was made by the UPBEAT study group to retain routine clinical measures and *exclude* anthropometry from further analyses to generate a more pragmatic model.

Table 27 summarises baseline comparisons of biomarkers in women with and without GDM following adjustment for age, parity [≥2], Black ethnicity, SBP and DBP.

Significant differences were observed for adiponectin only, with plasma concentrations 34% lower in women with GDM (95% CI [-47% to -19%], p<0.001). There was a trend towards significance for fructosamine in the GDM group (p=0.05), which did not persist after adjustment (p=0.82). No other biochemical markers were associated with GDM.

Plasma concentrations of AST and CRP were greater in the GDM group, although non-significant (17%, p=0.11 and 28%, p=0.18 respectively).

Table 27 Comparison of biomarkers by GDM status (adjusted for routinely used clinical predictors: age, parity [≥2], Black ethnicity, SBP and DBP)

Biomarker*	GDM (n=29)	No GDM (n=77)	Comparison (95% CI)	P value
Fructosamine (umol/l)	§200.87 (1.10)	192.90 (1.09)	1.00 (0.97 to 1.04)	0.82
ALT (U/L)	§21.41 (1.79)	19.00 (1.57)	1.12 (0.84 to 1.50)	0.42
AST (U/L)	§30.63 (1.53)	25.07 (1.41)	1.17 (0.96 to 1.43)	0.11
Ferritin (ng/ml)	§42.06 (2.27)	39.48 (2.29)	0.95 (0.64 to 1.41)	0.79
Adiponectin (µg/ml)	§4.97 (1.72)	7.34 (1.76)	0.66 (0.53 to 0.81)	<0.001
tPA (ng/ml)	§10.35 (1.49)	9.00 (1.47)	1.05 (0.86 to 1.28)	0.64
IL-6 (pg/ml)	¶1.01 (2.08)	•0.95 (2.54)	0.91 (0.66 to 1.24)	0.55
Leptin (pg/ml)	§53.82 (1.49)	◦59.36 (1.52)	0.92 (0.76 to 1.13)	0.44
Visfatin (ng/ml)	§4.94 (1.40)	◦5.28 (1.42)	0.93 (0.77 to 1.12)	0.42
Insulin (mU/l)	26.00 (2.99)	20.20 (2.78)	1.33 (0.80 to 2.21)	0.27
Cholesterol (mmol/l)	5.31 (1.18)	5.42 (1.21)	1.01 (0.93 to 1.10)	0.80
Triglycerides (mmol/l)	1.67 (1.42)	1.53 (1.38)	1.13 (0.96 to 1.32)	0.13
HDL (mmol/l)	1.64 (1.32)	1.71 (1.26)	0.94 (0.82 to 1.08)	0.39
CRP (mg/l)	9.18 (1.93)	7.77 (2.30)	1.28 (0.89 to 1.83)	0.18
VLDL (mmol/l)	0.76 (1.42)	0.71 (1.38)	1.13 (0.97 to 1.32)	0.12
LDL (mmol/l)	2.74 (1.39)	2.93 (1.34)	0.99 (0.86 to 1.14)	0.86
Cholesterol:HDL	3.23 (1.31	3.17 (1.27)	1.07 (0.95 to 1.21)	0.27
LDL:HDL	1.67 (1.56)	1.71 (1.45)	1.05 (0.87 to 1.27)	0.63

* indicates geometric means and ratios of geometric means

§n=28 and ¶n=27 •n=75 and ◦n=74

Only adiponectin predictive after allowing for major clinical variables.

In a combined logistic regression model including adiponectin and 5 clinical risk factors, the only consistent predictive variables were adiponectin (OR for a halving in adiponectin concentration 4.04 [95% CI 1.69 to 9.64], p=0.002) and maternal age (OR per additional year 1.18, [95% CI 1.04 to 1.34], p=0.01) (Table 28). Black ethnicity ceased to be predictive for the same reasons discussed in 5.5.1 Biomarker analysis, page 128.

Table 28 Combined logistic regression using significant biomarkers and routine clinical risk factors (age, parity [≥2], Black ethnicity, SBP, DBP and adiponectin)

	Odds Ratio	95% Confidence Interval	P value
Log adiponectin	0.13	0.04 to 0.47	0.002
Age (for each additional year)	1.18	1.04 to 1.34	0.01
Parity ≥2	2.09	0.50 to 8.73	0.31

Black ethnicity	1.35	0.42 to 4.33	0.62
SBP	1.04	0.95 to 1.13	0.41
DBP	1.08	0.98 to 1.19	0.15

5.5.2.1 Area under the curve-receiver operating curve (AUC-ROC)

An AUC-ROC of 0.760 [95% CI 0.645 to 0.875] for prediction of GDM was achieved with clinical predictors (age, parity, ethnicity and blood pressure) alone. The AUC-ROC increased significantly to 0.834 [95% CI 0.742 to 0.927] ($Ch^2(1)=4.00$, p=0.046) with addition of adiponectin (Figure 26).

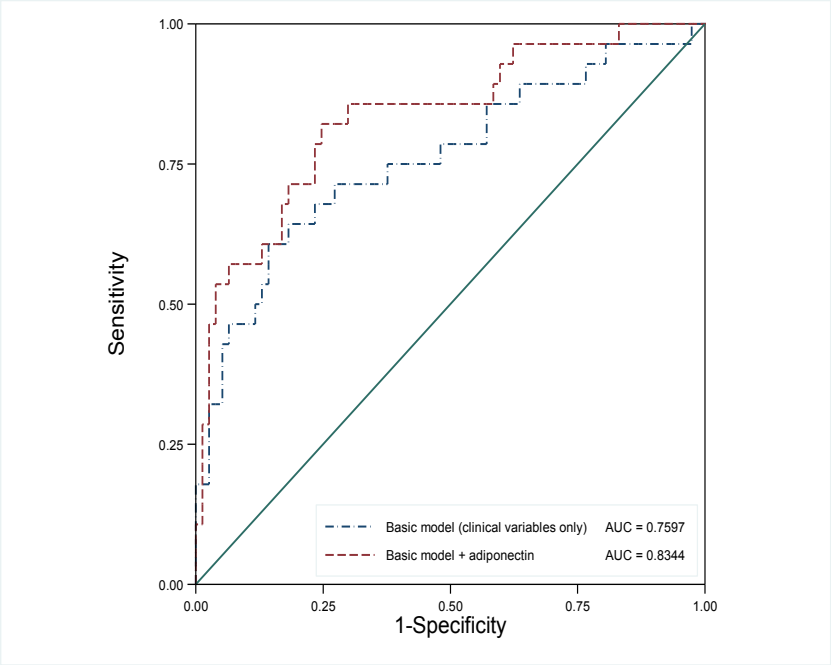


Figure 26 Receiver-operating curve and summaries using the basic model (including age, parity, ethnicity, blood pressure), with the addition of adiponectin. AUC, area under the receiver-operating curve.

6 RESULTS: IGPOP, A PILOT STUDY OF A SD-LGI DIETARY INTERVENTION

The increases in infant adiposity and metabolic complications following maternal obesity are in part a consequence of maternal insulin resistance and subsequent hyperglycaemia and dyslipidaemia (Dabelea, Hanson et al. 2000, Reynolds, Allan et al. 2013).

The ACHOIS and MFMU randomised controlled trials clearly demonstrated that treating IGT in pregnancy with dietary advice and insulin when needed, improves maternal and neonatal outcomes (Crowther, Hiller et al. 2005, Landon, Spong et al. 2009). It should be noted that although lowering the dietary glycaemic index (GI) of women in the intervention group in the Australian Carbohydrate Intolerance Study in Pregnant Women (ACHOIS) study was not a specific aim, it is likely that the overall GI was lowered as a result of the routine dietary advice given. For lean women with NGT in pregnancy, current evidence suggests potential benefits of LGI diets but the PREGGIO study (Moses, Casey et al. 2014) did not meet its primary aim of improving pregnancy outcome when diets of LGI and healthy eating were compared.

Nonetheless for obese pregnant women who have greater postprandial glycaemia (1hour and 2 hour) and reach a greater glucose peak (Yogev, Ben-Haroush et al. 2004, Harmon, Gerard et al. 2011), dietary modifications to reduce this exposure, when transfer of maternal glucose to the fetus is maximal, may yield favourable physiological and clinical outcomes.

Lowering the dietary GI and improving the quality of CHO consumed can blunt the postprandial glycaemic response and improve insulin sensitivity (Kwak, Paik et al. 2012). This includes switching from rapid to slow digesting carbohydrates and increasing the percentage contribution of resistant starch and indigestible fibres.

In this thesis, two nutritional supplement products (A and B) developed by Abbott Nutrition with the primary aim of blunting postprandial glycaemia were evaluated in obese pregnant women.

In stage one of the study the glycaemic response following a meal tolerance test (MTT) was determined, and the glycaemic index of prototype B in non pregnant lean women calculated following standard GI testing methodology (Brouns, Bjorck et al. 2005). In stage two, the prototype that displayed superior glucose lowering effects in stage 1 was tested to assess if the addition of a nutritional supplement in obese pregnant women improves glucose and insulin profiles when incorporated within a balanced diet. Methods are described in detail in Chapter 4, Methods: Improving Glycaemic Profiles in Obese Pregnancies (IGPOP), page 91.

6.1 Stage 1a

Stage 1a investigated the effects of 2 slow-digesting LGI (SD-LGI) nutritional supplements (A and B) on postprandial glycaemia versus a control (D) in 4 categories of women. Capillary blood glucose was measured at 9 time points up to 240 minutes and the incremental area under the curve (iAUC) calculated.

The nutritional composition of the three products is detailed in Dietary composition of nutritional supplements, page 91.

Ten women were recruited to each of the 4 groups: lean non-pregnant (LNP), lean pregnant (LP), obese non-pregnant (ONP) and obese pregnant (OP). Gestational age was 24^{+0}-28^{+6} weeks' for the pregnant women. The mean BMI with ethnicity for each group is given below.

Table 29 Demographic details of the four groups of women participating in stage 1a

Category (n=10)	Mean BMI (kg/m^2)	Age (years)	Ethnicity
Lean non-pregnant (LNP)	22.5 (1.5)	25.6 (4.1)	European: 7
			Indian: 1
			Chinese: 2
Lean pregnant (LP)	22.1 (1.6)	27.9 (3.8)	European: 4
			Black: 6
Obese non-pregnant (ONP)	35.3 (4.9)	28.4 (5.4)	European: 6
			Black: 3
			Mixed: 1
Obese pregnant (OP)	38.5 (6.7)	32.5 (4.9)	European: 4
			Black: 6

*BMI and age given as mean (SD), ethnicity given as (n).

Table 30 summarises the FBG and iAUC for all three supplements in the four categories of women. For each group, B yielded the lowest iAUC when compared to A and D respectively.

With equivalent caloric value and carbohydrate content, the only difference in macronutrient composition between A and B was fat concentration with B containing a high fat load (14g v 1g per 16oz serving).

Table 30 Fasting blood glucose concentration and iAUC for the three test supplements in all subjects

Study group	**A**	**B**	**D**
Lean non-pregnant: LNP			
FBG	4.10 (0.15)	4.41 (0.19)	4.65 (0.21)
iAUC	166.7 (20.4)	72.5 (12.3)	200.4 (28.8)
Lean pregnant: LP			
FBG	4.32 (0.12)	4.14 (0.10)	4.11 (0.09)
iAUC	101.5 (10.2)	95.8 (14.1)	253.2(14.7)
Obese non-pregnant: ONP			
FBG	4.69 (0.11)	4.74 (0.09)	4.75 (0.09)
iAUC	130.1 (26.8)	103.9 (12.3)	226.0 (40.4)
Obese pregnant: OP			
FBG	4.47 (0.14)	4.62 (0.12)	4.69 (0.17)
iAUC	184.3 (23.7)	181.5 (23.0)	259.1 (30.8)

FBG: fasting blood glucose given as mean (SEM) in mmol/l. iAUC: incremental area under the curve given as mean (SEM) in mmol/l/240min

In keeping with IR of pregnancy, compounded by obesity, LNP women had the lowest iAUC (72.5 mmol/l/240min), which progressively increased in the order of LP, ONP and OP to a maximum of 181.5mmol/l/240min following consumption of B. This pattern was less consistent for A and D.

One-way ANOVA test with Tukey's multiple comparison analysis confirmed a consistent reduction in iAUC for B versus D (control) across all groups notably in the obese pregnant group. No significant differences were found between the iAUC for A and B (Table 31).

Table 31 Comparison between A, B and D within each study group

One way ANOVA (Tukey's multiple comparison test)	**Mean Difference of iAUC per participant**	**P value**	**95% CI of difference**
Lean non-pregnant (LNP)			
A vs B	0.42	0.08	-0.04 to 0.87
A vs D	-0.18	0.06	-0.63 to 0.28
B vs D	-0.59	0.002	-1.05 to -0.14
Lean pregnant (LP)			
A vs B	-0.06	0.56	-0.67 to 0.55
A vs D	-0.79	0.004	-1.39 to -0.18
B vs D	-0.73	0.004	-1.33 to -0.12
Obese non-pregnant (ONP)			
A vs B	0.06	0.38	-0.44 to 0.55
A vs D	-0.44	0.03	-0.94 to 0.05
B vs D	-0.50	0.01	-0.99 to -0.00
Obese pregnant (OP)			
A vs B	0.25	0.38	-0.18 to 0.67
A vs D	-0.26	0.08	-0.69 to 0.17
B vs D	-0.51	0.03	-0.93 to -0.08

When comparing the glucose response of supplement B across the four categories of women, the iAUC was greatest in the obese pregnant group compared to lean and non-pregnant women.

The timing of the post-prandial peak for all supplements was comparable at approximately 60 minutes with the greatest increment recorded for D on each occasion (Figure 27 to Figure 30).

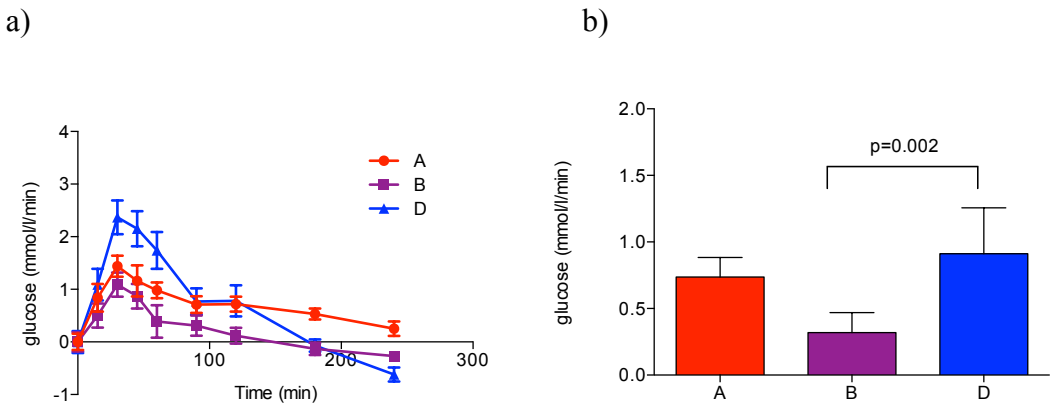


Figure 27 a) Line graph and b) box plot of glucose iAUC lean non pregnant (LNP) women for A, B & D (n=10). Error bars represent mean± SEM

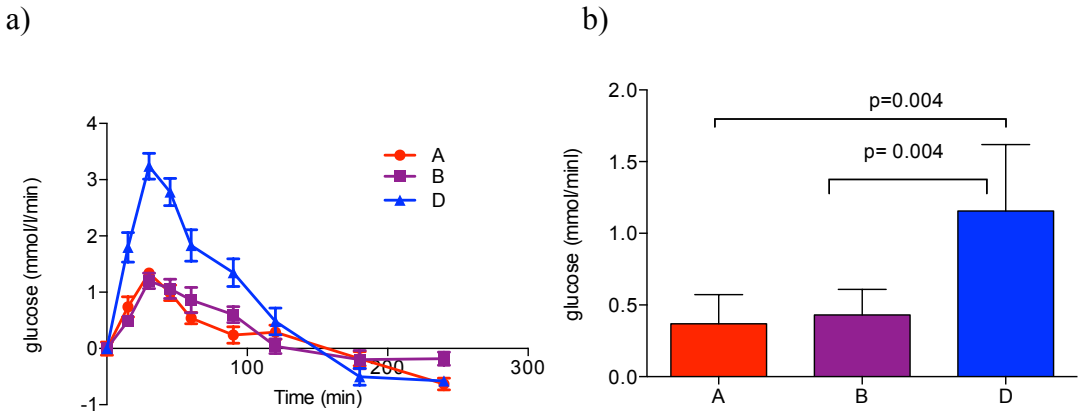


Figure 28 a) Line graph and b) box plot of glucose iAUC for lean pregnant (LP) women for A, B & D (n=10). Error bars represent mean± SEM.

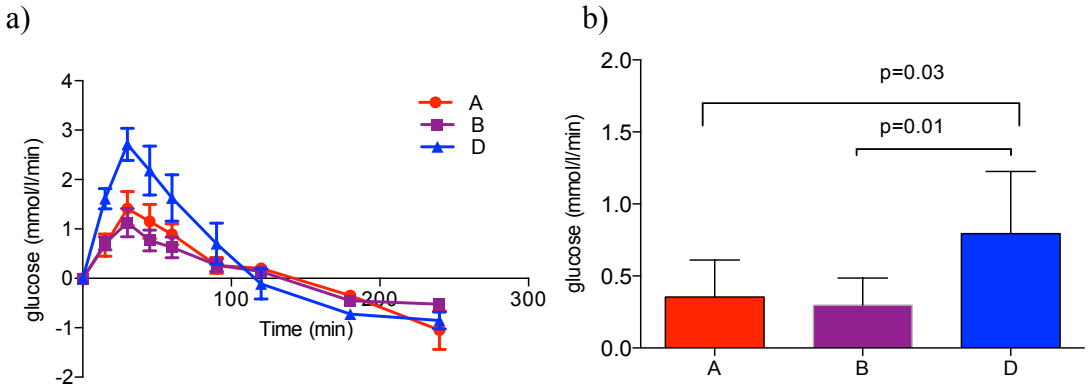


Figure 29 a) Line graph and b) box plot of glucose iAUC for obese non pregnant (ONP) women for A, B & D (n=10). Error bars represent mean± SEM.

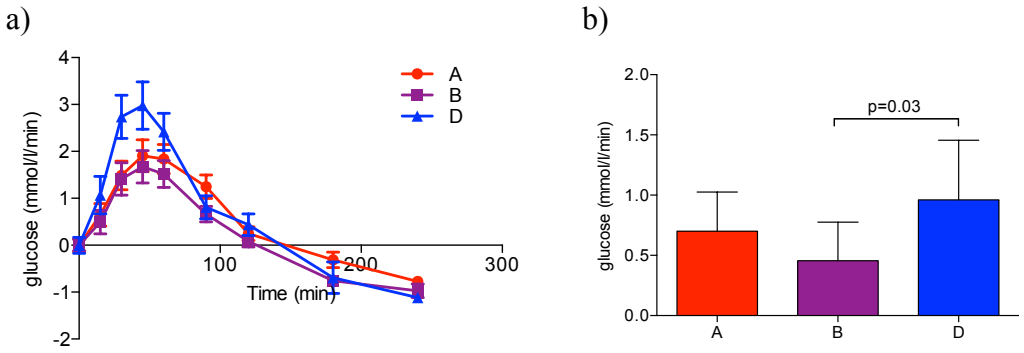


Figure 30 a) Line graph and b) box plot of glucose iAUC for obese pregnant (OP) women for A, B & D (n=10). Error bars represent mean± SEM.

6.1.1 Stage 1a palatability

All participants completed online palatability questionnaires during each of the three tests. Overall satisfaction for B was high with 17 women stating that they "liked it" compared to 7 and 14 positive replies for supplements A and D respectively. Dissatisfaction was greatest for A as shown in Figure 31.

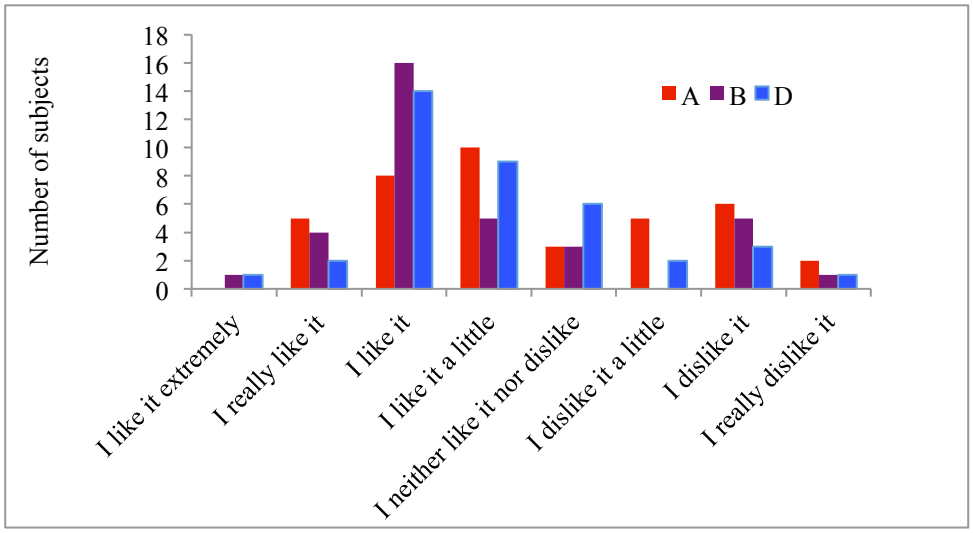


Figure 31 Summary of stage 1a palatability findings for all women combined (LNP, LP, ONP, and OP)

There was no difference in terms of general acceptability in pregnant women when asked the question "*how likely are you to drink the product?"* There was however a clear indication that women would be prepared to consume a nutritional drink during pregnancy if advised by a member of their healthcare team (Figure 32).

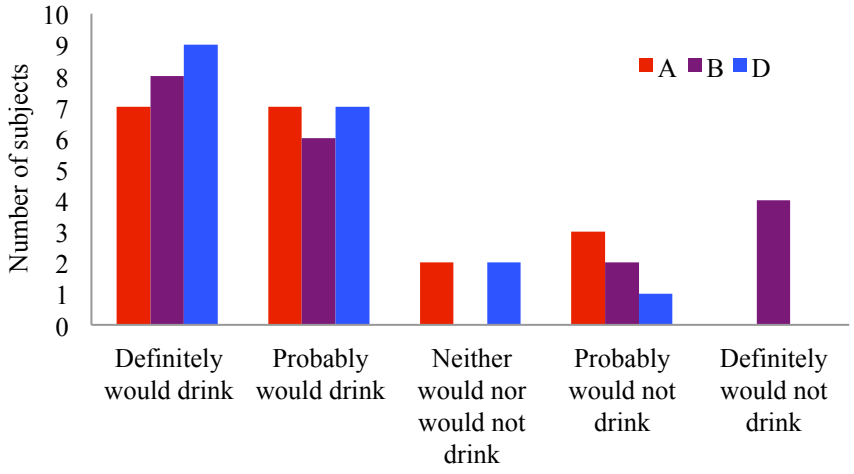


Figure 32 Histogram of responses to the question "how likely are you to drink the product?" in lean and obese pregnant women combined

Furthermore, pregnant women were more likely to consider consuming more servings per day compared to non-pregnant women: (A) 2.47 v 1.81, (B) 2.75 v 2.0 and (D) 2.53 v 1.83 (pregnant versus non-pregnant).

6.1.2 Summary of Stage 1a

In summary, B led to the lowest iAUC compared to A and D in all categories of women. This was associated with high scores for palatability particularly in pregnant women who would consider consuming the drink during pregnancy if recommended. On the basis of these findings, it was concluded that supplement B was most likely to confer benefit in terms postprandial glucose excursions and was therefore investigated in stage 2 which focussed on obese pregnant women.

6.2 Stage 1b

To determine the GI of B, two 50g glucose challenge tests were performed, in 10 lean women. CBG was measured at 7 time points up to 120 minutes (0, 15, 30, 45, 60, 90 and 120 minutes), to provide 50g CHO, the following volumes were given:

1) Supplement B: 257ml and
2) Standard: 273ml of Lucozade Original® (70kcal/100ml)

Incremental AUC for Lucozade and B was 13.00 and 3.56 respectively. Using the standard formula shown below, the GI of B was 27.38, confirming its low GI classification (low ≤50, medium 56-69 and high ≥70) (Wolever, Jenkins et al. 1991).

Table 32 Summary of outcome measures for stage 1b. Data is presented a mean (SD) for baseline and peak glucose concentration. *SD not available for iAUC

	Lucozade (n=10)	**B (n=10)**	**GI value of the supplement**
Baseline glucose (mmol/l)	4.54 (0.54)	4.27 (0.50)	=(iAUC B/iAUC standard)*100
Peak glucose (mmol/l)	8.09 (1.05)	5.42 (0.59)	= (66.22/237.5)*100
iAUC (mmol/l/min)*	237.5	66.22	=**27.38**

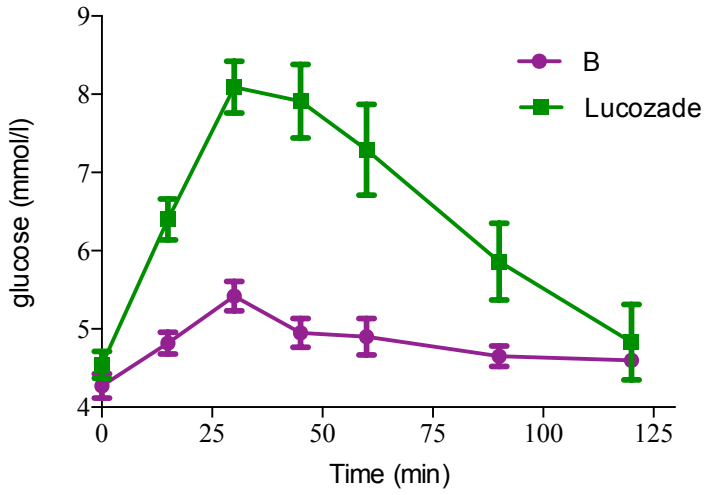


Figure 33 Line graph of mean capillary glucose concentrations in stage 1b for lean non pregnant women (n=10) following consumption of supplement B (•) and the standard glucose control Lucozade (■). Error bars represent mean± SEM.

7 RESULTS: IGPOP STAGE 2

Stage 2 was a single blinded randomised crossover design in obese pregnant women. The aim was to evaluate the immediate and extended effects on glucose and insulin concentrations, following consumption of the control (D) or intervention (B) nutritional supplements as part of a controlled diet using CGMS.

Macronutrient content of the two products was equivalent (CHO 60.7%, fat 20.8% and protein 18.5%) with differences in CHO composition of the two supplements summarised below. Detailed composition is provided in Chapter 4.1, page 91.

Table 33 Macronutrient composition of intervention supplement (B) and control (D) per 16oz serving used in Stage 2

Macronutrient	B (intervention)	D (control)
Slow digesting carbohydrates (%)	68	-
Rapid digesting carbohydrates (%)	5	100
Resistant starch (%)	15.5	-
Indigestible fibre (g)	3.5	-
Fat (g)	14.0	14.0
Calories per 16oz (474ml) serving (Kcal)	303	303

The study was divided into three distinct 48hour periods following randomisation to the intervention or control on the 1st visit to the CRF:

1. Thursday* and Friday•: intervention or control
2. Saturday and Sunday: washout with habitual diet
3. Monday* and Tuesday•: intervention or control

*Denotes study day at CRF and • study day at home with ongoing CGMS

Of the 25 women recruited 3 were excluded. Two had multiple problems with sensor failure despite re-insertion of new devices and one was excluded following preliminary review of the CGMS data, which confirmed non-compliance with the prescribed diet.

As described in detail below, sixteen women were randomly selected from the sample of 22 (ID numbers 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 15, 16, 18, 19 and 21)

with 8 subjects for each sequence of treatment as described in Chapter 4.5.2, page 110.

The mean age of the sample was 31 years (range 21-39, SD 4.8) and BMI 37kg/m^2 (range 31-46, SD 4.7). Three quarters of women were of black ethnic origin.

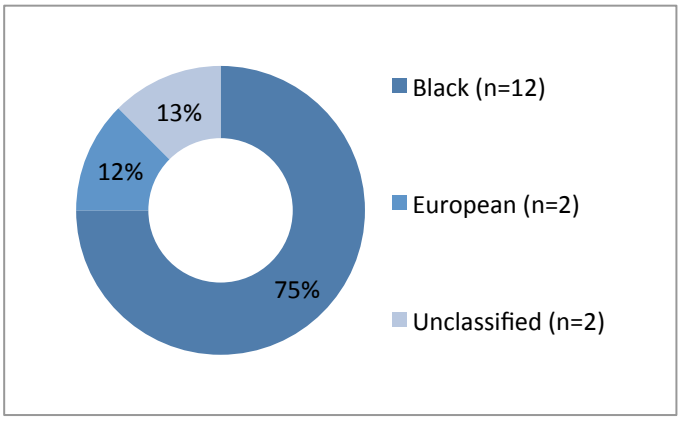


Figure 34 Pie chart of ethnicity of the selected sample (n=16)

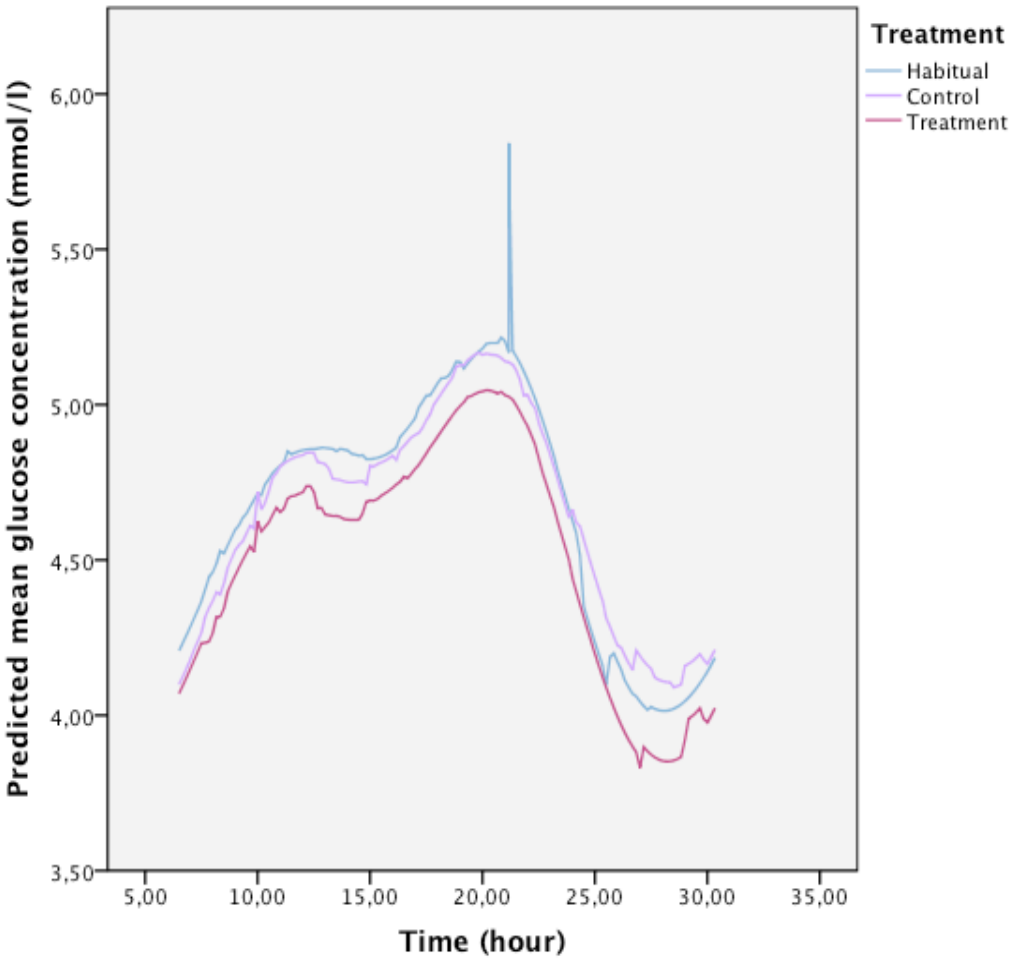
7.1 CGMS

A model based on individual observations instead of summary statistics, was fitted to the data. The effect of treatment over time was then analysed using linear mixed models adjusted by period and day.

The following tables show the parameter estimates from fitting the linear mixed model (LMM) to the observed data points every ten minutes.

7.1.1 24hr glucose estimates

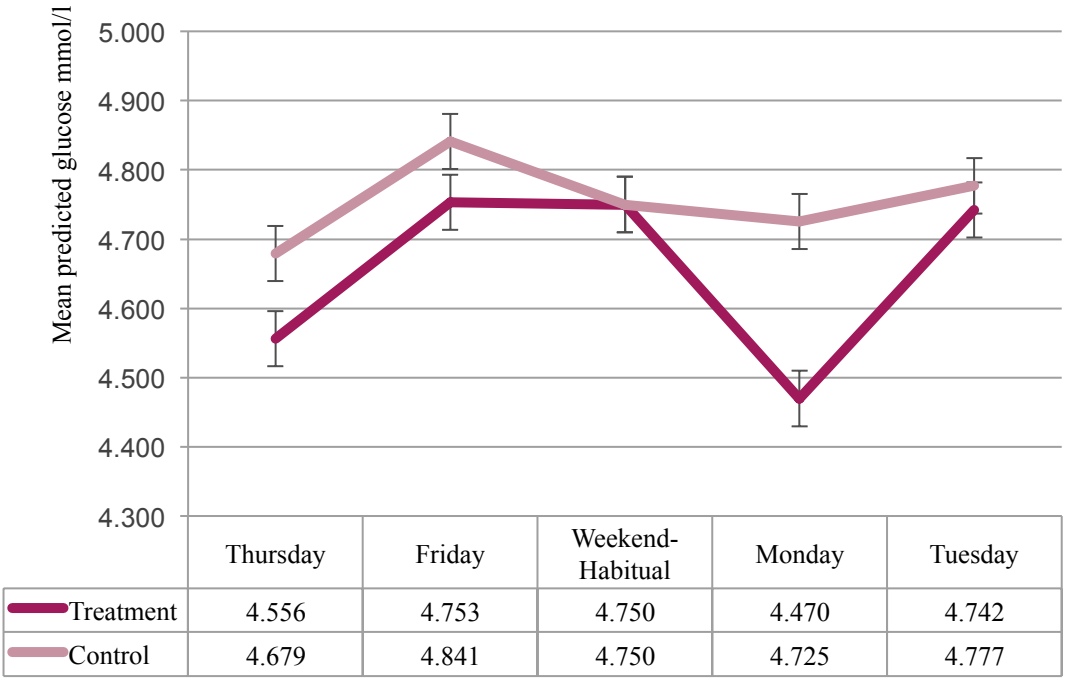
1. When considering the overall performance of the supplement treatment throughout the study (over 2 days and one night) the glucose curve derived from the LMM was lower than both habitual and control as shown in Figure 35. Furthermore the glucose estimates for the habitual diet and control days were significantly greater with values of 0.27mmol/l and 0.23mmol/l respectively ($p<0.001$ for both).



	Estimates of fixed effects			
Parameter	**Estimate (mmol/l)**	**Standard error**	**P Value**	**95% CI**
Habitual v treatment	0.27	0.03	<0.001	0.21 to 0.32
Control v treatment	0.23	0.04	<0.001	0.16 to 0.31
Hospital v home	-0.02	0.03	0.39	-0.07 to 0.03

Figure 35 Graph with summary of estimates showing the overall effect in glucose concentration for the intervention supplement compared to the control and habitual period (2 days and 1 night) following LMM. Estimated glucose concentrations in mmol/l. The x-axis represents 24 hours following consumption of the intervention/control supplement using combined data for each 2-day test period following LMM.

2. A direct comparison between control and intervention is shown in Figure 36. Greater differences were noted on hospital research days (Thursday and Monday) compared to those at home (Friday and Tuesday). However the differences remained significant between intervention and control excluding the final day.



	Thursday	Friday	Weekend-Habitual	Monday	Tuesday
Treatment	4.556	4.753	4.750	4.470	4.742
Control	4.679	4.841	4.750	4.725	4.777

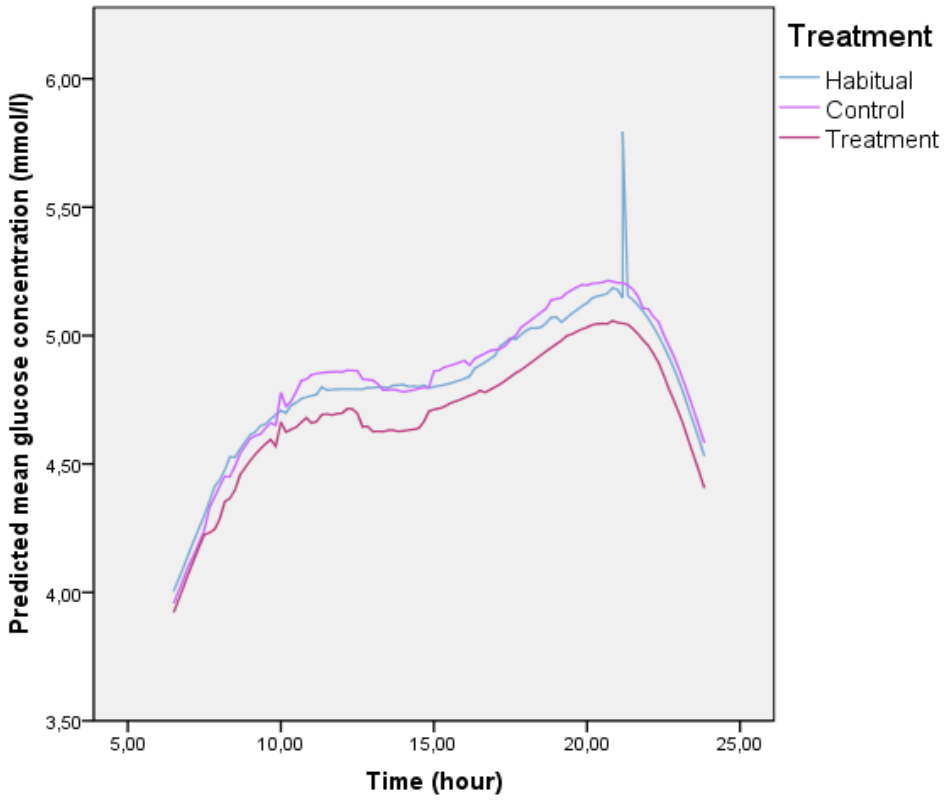
	Treatment (mmol/l)	Control (mmol/)	P Value
Thursday	4.56 (4.51-4.61)	4.68 (4.63-4.72)	<0.001
Friday	4.75 (4.71-4.79)	4.84 (4.81-4.88)	0.001
Weekend			
Monday	4.47 (4.43-4.51)	4.73 (4.68-4.77)	<0.001
Tuesday	4.74 (4.71-4.78)	4.78 (4.74-4.82)	0.51

Figure 36 Graph and estimates of glucose concentrations for each study day showing direct comparison between intervention and control. Data presented as mean glucose (mmol/l [range]) and bars represent the 95% confidence interval.

7.1.2 Daytime glucose observations

The overall comparison of the treatment compared to the control is shown in Figure 37 with corresponding estimates plotted for daytime observations only.

Predicted mean blood glucose concentrations were consistently lower for the treatment throughout the day with significantly greater estimates for habitual and control diets ($p<0.001$ for comparison of treatment with habitual and control). No difference was observed between hospital versus home study days.



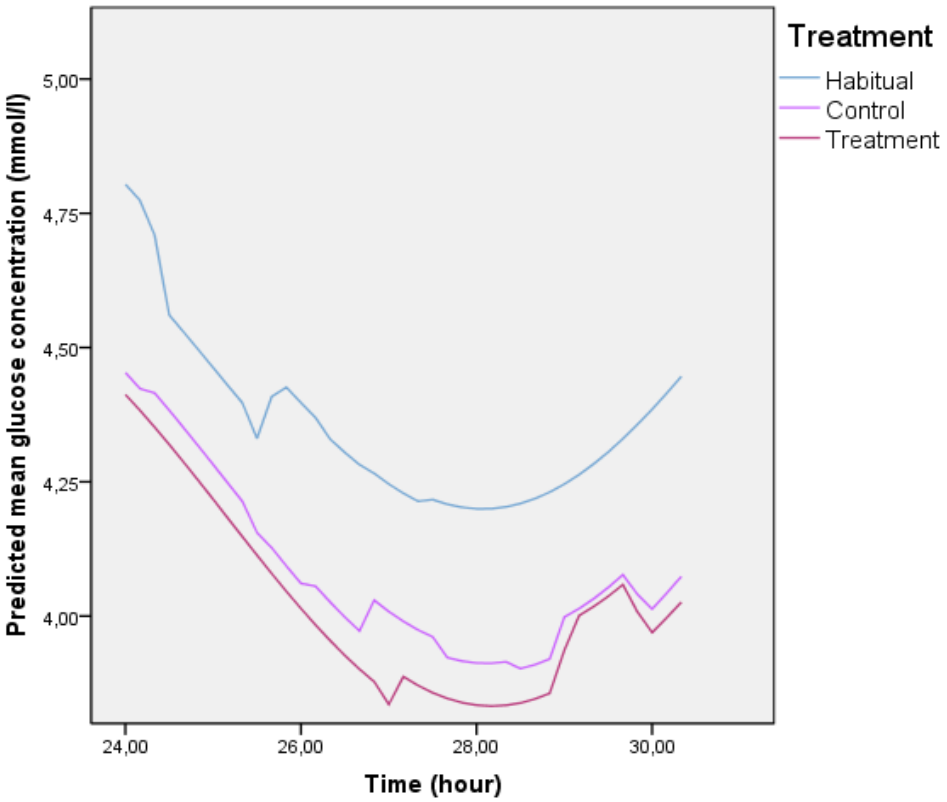
	Estimates of fixed effects			
Parameter	**Estimate (mmol/l)**	**Standard error**	**P Value**	**95% CI**
Habitual v treatment	0.25	0.03	<0.001	0.19 to 0.31
Control v treatment	0.26	0.04	<0.001	0.18 to 0.34
Hospital v home	-0.02	0.03	0.54	-0.08 to 0.04

Figure 37 Graph with summary of estimates for nocturnal glucose concentrations in mmol/l following LMM for the intervention, control and habitual periods. The x-axis represents 24 hours following consumption of the intervention/control supplement using combined data for each 2-day test period following LMM.

7.1.3 Nocturnal glucose observations

Data for the second night was discarded for clinical reasons, therefore analysis included the first night for each two-day period.

The overall response curve was lower following the intervention supplement compared to the control but no significant difference was found (p=0.09). Glucose estimates during the habitual period however were significantly greater when compared to the intervention (p<0.001) (Figure 38).



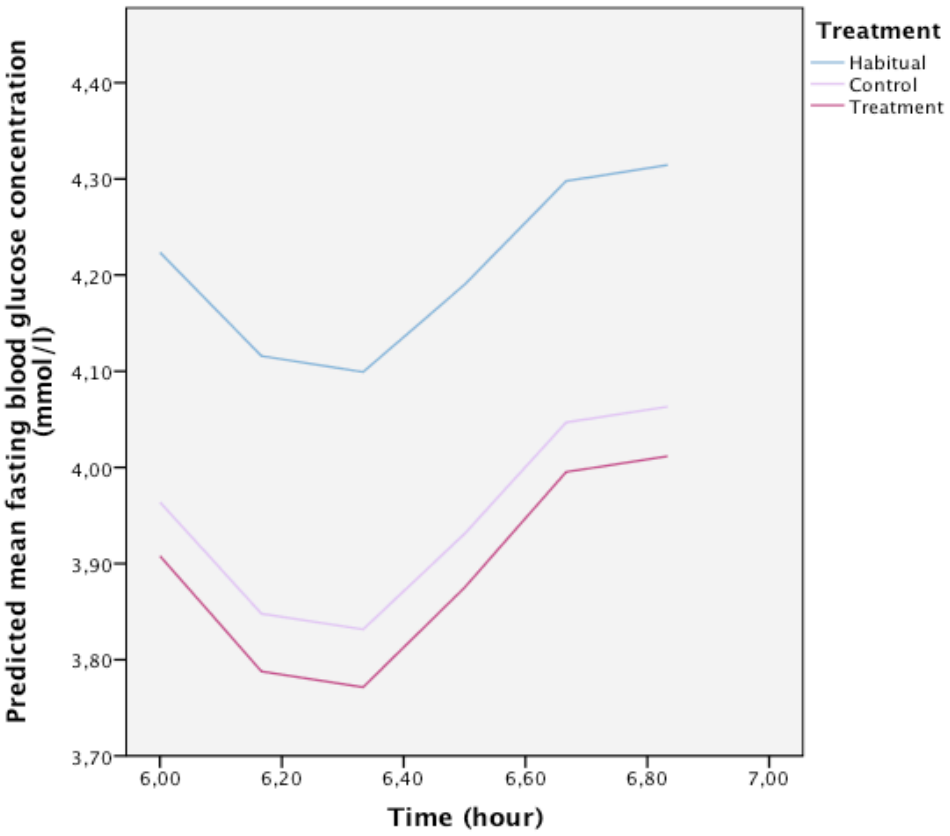
	Estimates of fixed effects			
Parameter	**Estimate (mmol/l)**	**Standard error**	**P Value**	**95% CI**
Habitual v treatment	0.36	0.03	<0.001	0.29 to 0.42
Control v treatment	0.05	0.04	0.09	-0.01 to 0.11

Figure 38 Graph with summary of estimates for nocturnal glucose concentrations in mmol/l following LMM for the intervention, control and habitual periods.

7.1.4 Fasting blood glucose

A LMM was fitted to the 6 consecutive glucose measures recorded between 0600hr and 0650hr with data included from one day of each phase. A reduction in glucose concentration was seen in all groups until approximately 0620hr followed a progressive rise thereafter.

Glucose concentrations for the treatment were significantly lower compared to habitual data ($p<0.001$) but no different to the control ($p=0.22$) (Figure 39).



		Estimates of fixed effects		
Parameter	**Estimate (mmol/l)**	**Standard error**	**P Value**	**95% CI**
Habitual v treatment	0.41	0.07	<0.001	0.28 to 0.55
Control v treatment	0.08	0.06	0.22	-0.04 to 0.19

Figure 39 Graph with summary of estimates for fasting glucose concentrations in mmol/l following LMM for the intervention, control and habitual periods.

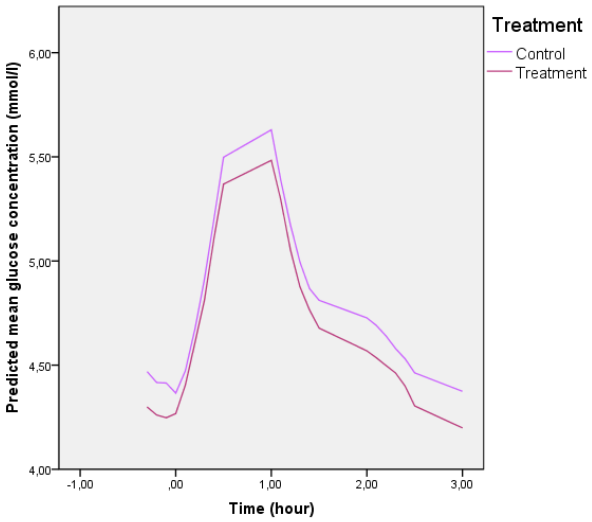
7.1.5 Postprandial glucose

Postprandial data for treatment and control (30 minutes pre-meal to 180 minutes post) was analysed for study days only. During the habitual period, women were not requested to record meal markers.

Breakfast was the only meal where a significant difference in postprandial glycaemia was observed with greater glucose concentrations following the control (p=0.03, 0.34 and 0.71 for breakfast, lunch and dinner respectively).

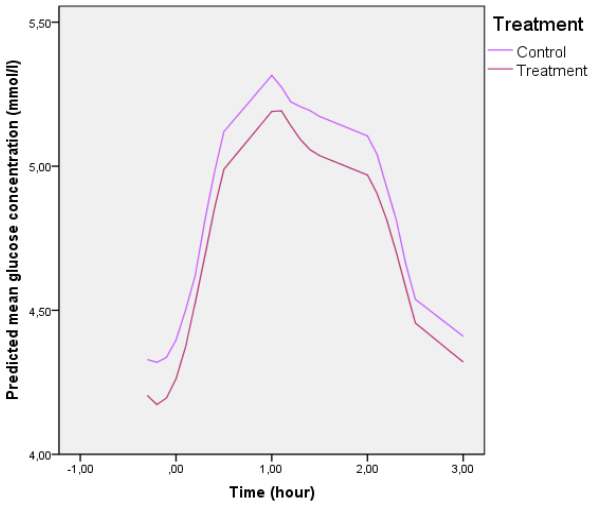
Postprandial glucose concentrations were generally lower on hospital days compared to the second day at home for all meals in both arms (breakfast p<0.001, lunch p=0.80 and dinner p=0.43).

Graphs representing the postprandial glycaemic response for each meal with summaries of estimates for glucose concentrations following LMM are shown (Figure 40 to Figure 42).



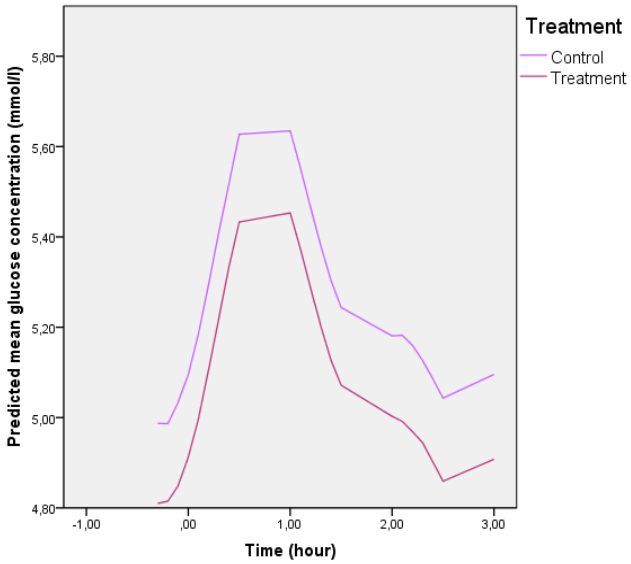
	Estimates of fixed effects			
Parameter	**Estimate (mmol/l)**	**Standard error**	**P Value**	**95% CI**
Control v treatment	0.09	0.04	0.03	0.01 to 0.18
Interaction: home v hospital	-0.58	0.05	<0.001	-0.68 to -0.49

Figure 40 Graph with summary of estimates for breakfast postprandial glucose concentrations (180minutes) in mmol/l following LMM for the intervention versus control.



	Estimates of fixed effects			
Parameter	**Estimate (mmol/l)**	**Standard error**	**P Value**	**95% CI**
Control v treatment	-0.20	0.21	0.34	-0.62 to 0.22
Interaction: home v hospital	0.06	0.21	0.80	-0.37 to 0.48

Figure 41 Graph with summary of estimates for lunch postprandial glucose concentrations (180minutes) in mmol/l following LMM for the intervention versus control.



	Estimates of fixed effects			
Parameter	**Estimate (mmol/l)**	**Standard error**	**P Value**	**95% CI**
Control v treatment	-0.20	0.06	0.71	-0.13 to 0.09
Interaction: home v hospital	-0.05	0.06	0.43	-0.16 to 0.07

Figure 42 Graph with summary of estimates for dinner postprandial glucose concentrations (180minutes) in mmol/l following LMM for the intervention versus control.

7.2 IGPOP biochemistry: Insulin, C-Peptide, NEFA and Triglycerides

Samples were obtained up to 210 minutes following consumption of the intervention (B) and control (D) supplements for all subjects participating in the study but only data for the same 16 women included in the CGMS analysis was used. Results were recorded at 5 and 15 time points for NEFA/triglycerides and insulin/C-peptide respectively with logarithmic transformation performed for insulin and C-peptide only, following standard distributional checks. Tables reporting the mean concentration of each biomarker at each time point with 95% CI are included in Appendix 3: IGPOP Biochemistry Results, page 214.

For all biomarkers, a difference was observed between women depending on the sequence order they were allocated to, following simple randomisation on day 1 (sequence 1 intervention/control and sequence 2 control/intervention). After adjustment for repeated measures, this difference was not significant however and the patterns of response were attributed to chance alone. Figures for each biomarker

are presented below initially by study visit number to illustrate the influence of the sequence order, followed by a line graph of combined data, summarising potential differences between the intervention and control over the duration of the study (Figure 43 to Figure 50).

Linear regression analysis found no detectable effect of the intervention supplement (B) when compared to the control for insulin, C-peptide and triglycerides (Table 34). A borderline significant trend towards greater concentrations of plasma NEFA following the intervention supplement was observed (p=0.049).

Table 34 Results of linear regression analysis to examine for any effect or difference in plasma concentrations of insulin, C-peptide, NEFA and triglycerides following consumption of the test (B) compared to the control (D) in obese pregnant women (n=16)

Biomarker	Treatment effect	95% CI	P value
	Ratio of geometric means		
Insulin	0.98	0.88 to 1.09	0.68
C-peptide	0.97	0.92 to 1.02	0.29
	Difference in arithmetic mean		
NEFA	0.05	0.00 to 0.10	0.049
Triglycerides	0.04	-0.01 to 0.10	0.15

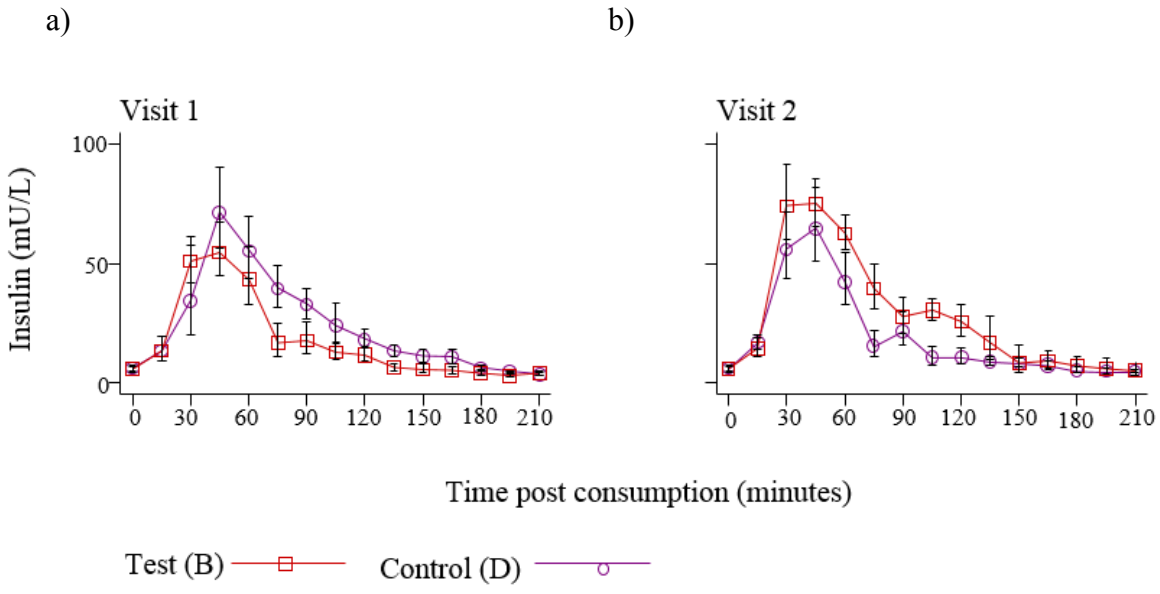


Figure 43 Plasma concentration of insulin (mU/L) 210 minutes post consumption of intervention (B) and control (D) on a) visit 1 and b) visit 2 to the CRF for n=16 obese pregnant women. Data presented as geometric mean±SEM.

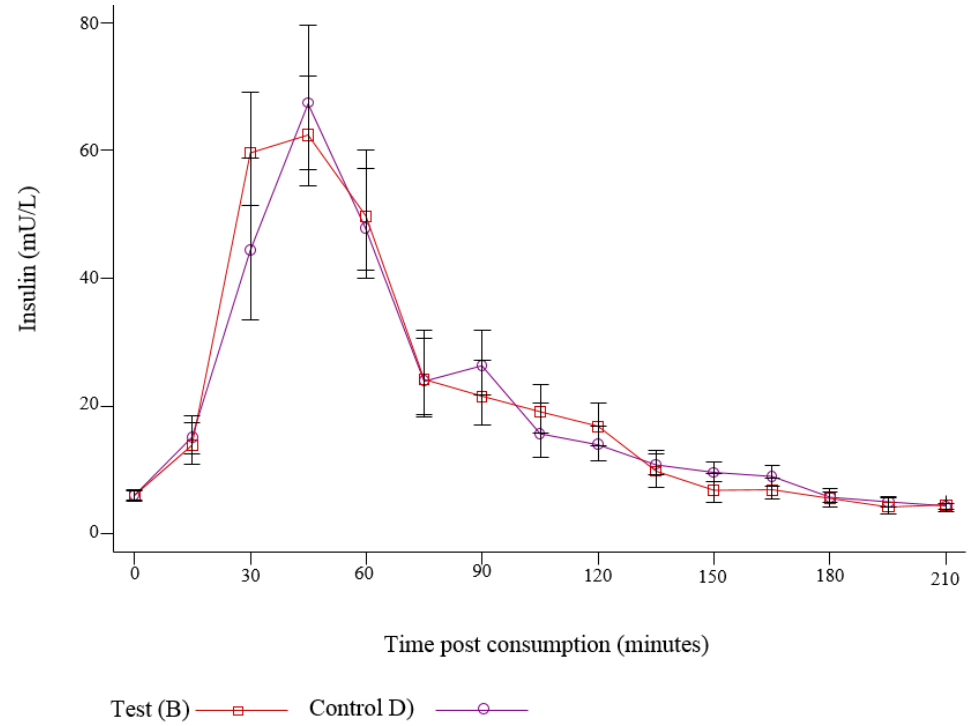


Figure 44 Combined data of visits 1 and 2 for plasma insulin concentration (mU/L) measured 210 minutes post consumption of intervention (B) and control (D) in 16 obese pregnant women. Data presented as geometric mean±SEM.

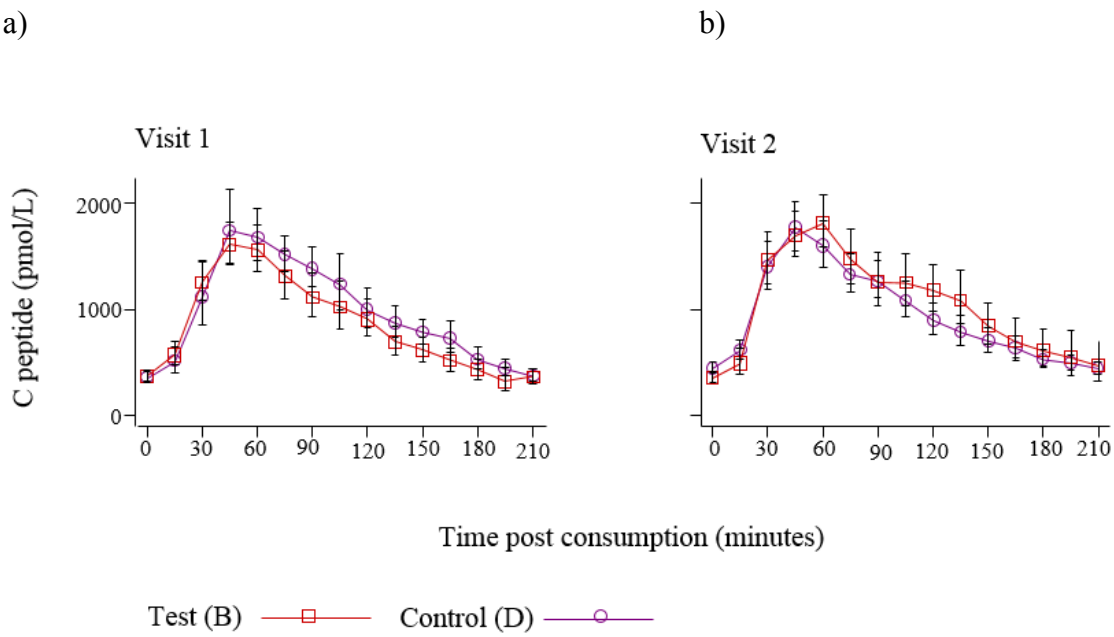


Figure 45 Plasma concentration of C-peptide (pmol/L) 210 minutes post consumption of intervention (B) and control (D) on a) visit 1 and b) visit 2 to the CRF for n=16 obese pregnant women. Data presented as geometric mean±SEM

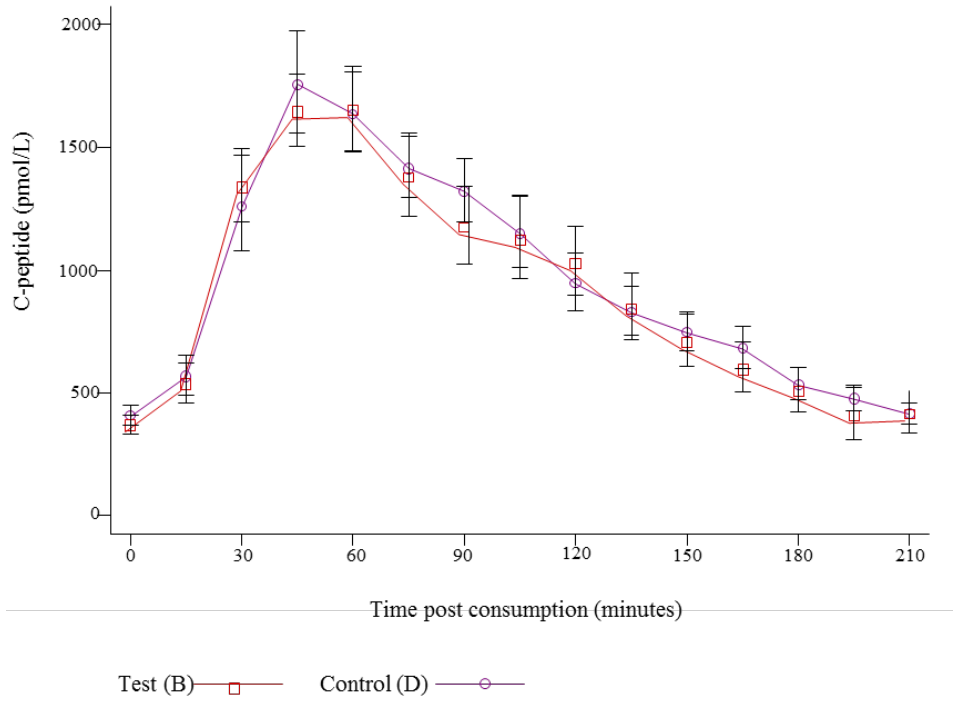


Figure 46 Combined data of visits 1 and 2 for plasma C-peptide concentration (pmol/L) measured 210 minutes post consumption of intervention (B) and control (D) in 16 obese pregnant women. Data presented as geometric mean±SEM.

a) b)

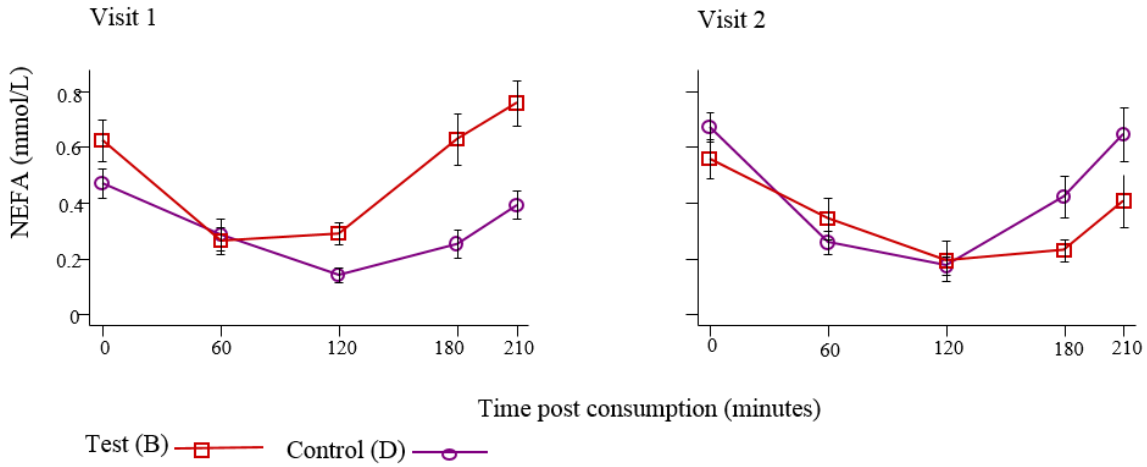


Figure 47 Plasma concentration of NEFA (mmol/L) 210 minutes post consumption of intervention (B) and control (D) on a) visit 1 and b) visit 2 to the CRF for n=16 obese pregnant women. Data presented as mean±SEM.

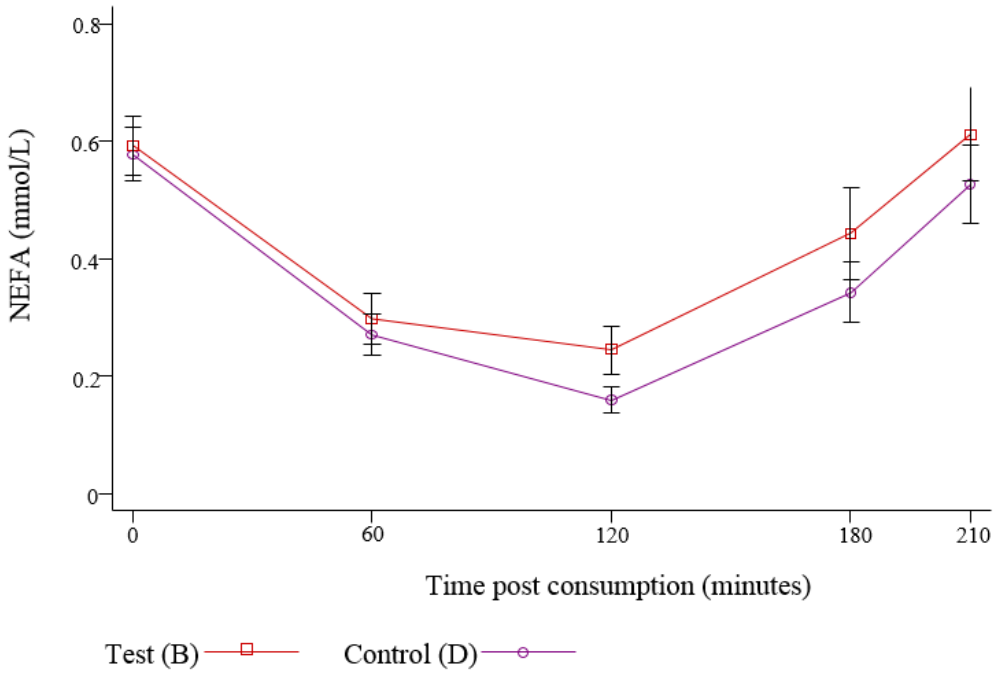


Figure 48 Combined data of visits 1 and 2 for plasma NEFA concentration (mmol/L) measured 210 minutes post consumption of intervention (B) and control (D) in 16 obese pregnant women. Data presented as mean±SEM.

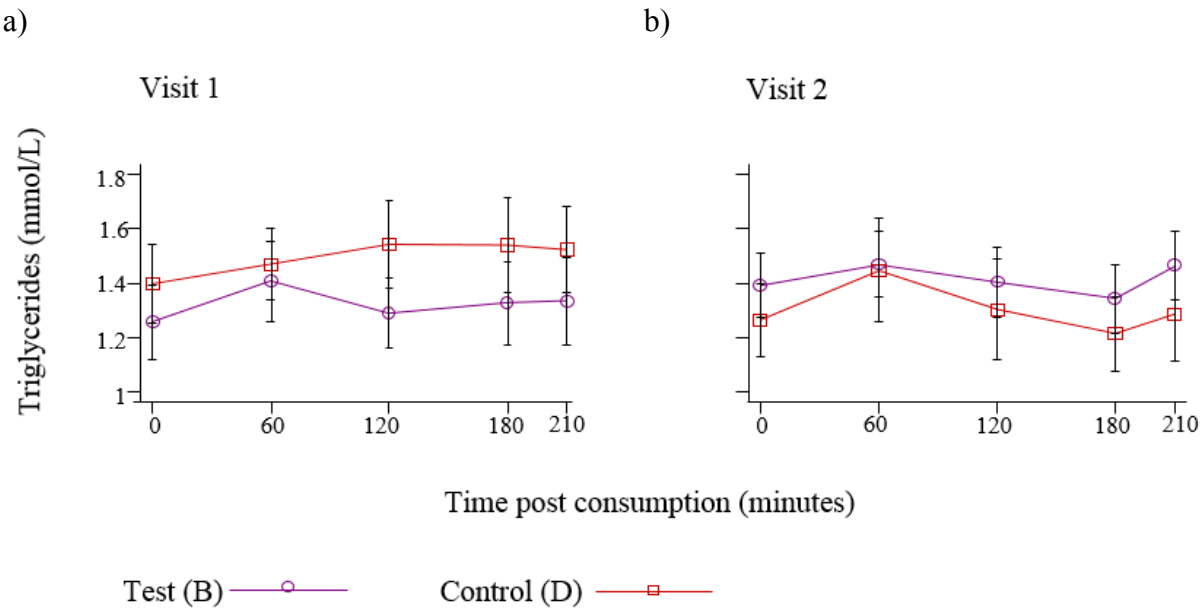


Figure 49 Plasma concentration of triglycerides (mmol/L) 210 minutes post consumption of intervention (B) and control (D) on a) visit 1 and b) visit 2 to the CRF for n=16 obese pregnant women. Data presented as mean±SEM.

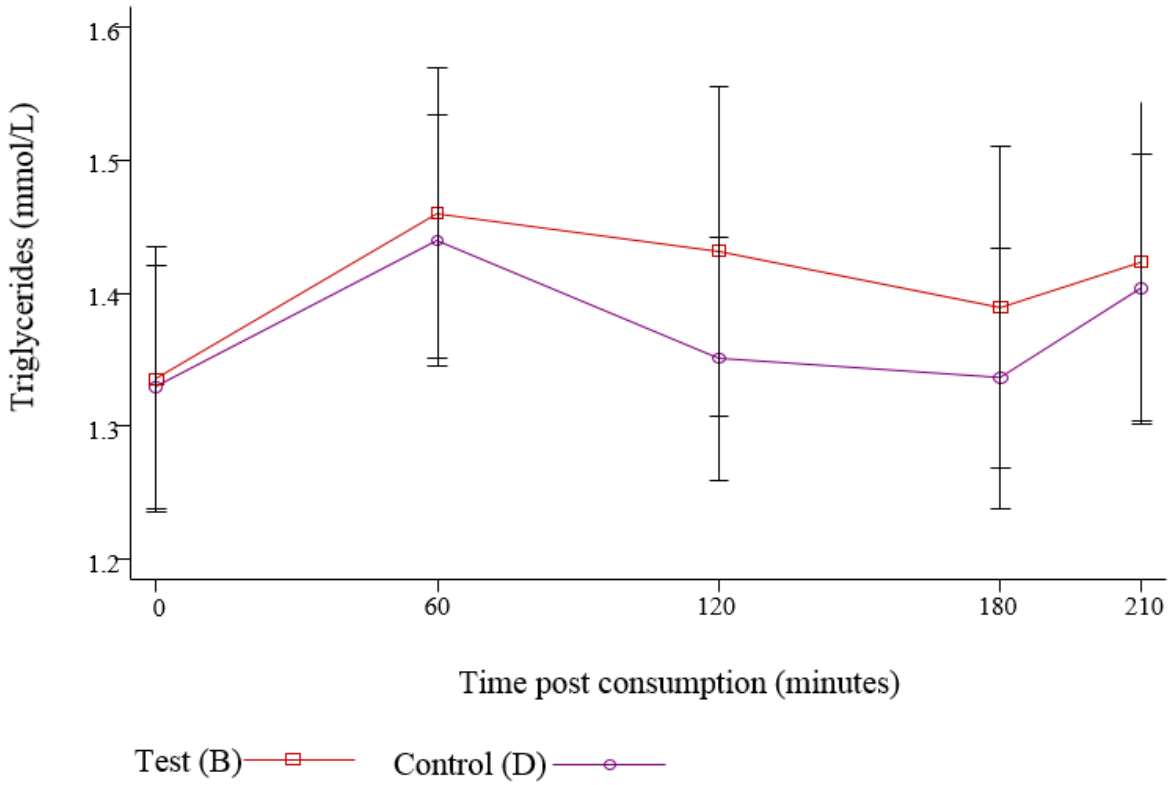


Figure 50 Combined data of visits 1 and 2 for plasma triglyceride concentration (mmol/L) measured 210 minutes post consumption of intervention (B) and control (D) in 16 obese pregnant women. Data presented as geometric mean±SEM.

8 DISCUSSION

The importance of lifestyle modifications *before* pregnancy to improve clinical outcomes, particularly GDM, cannot be underestimated as demonstrated by Zhang et al. who studied a prospective cohort of 14, 437 nurses participating in the Nurses' Health Study II (Zhang, Tobias et al. 2014). All factors (BMI, diet, activity and smoking status) were independently associated with GDM but BMI was the strongest risk factor following adjustment. Conversely the combination of 4 risk factors deemed to be of lowest risk (BMI<25kg/m^2, non-smoker, healthy eating and adequate exercise) was associated with a 52% reduced risk of GDM.

CGMS technology has revealed important aberrations in glucose homeostasis, notably a delayed and greater post prandial peak glucose concentration, specifically in obese women who may not meet current criteria for GDM, (Yogev, Ben-Haroush et al. 2004). Furthermore, positive associations between greater fasting and postprandial glucose concentrations and increased fetal fat mass have been identified in these "non-GDM" women (Harmon, Gerard et al. 2011). Considering the CGMS data, together with confirmation of abnormal pregnancy outcomes at lower glucose thresholds, targeting glycaemic control and evaluating influences of interventions on markers of insulin resistance and adipocyte function in obese pregnant women at risk of GDM as in UPBEAT are obviously justifiable. This is the rationale which has underpinned the studies in this thesis.

8.1 The influence of the UPBEAT intervention on biomarkers of insulin resistance and biomarkers of adipocyte function: a pilot study

The first study in the thesis was to address the influence of the dietary and physical activity intervention of UPBEAT on a range of biomarkers implicated in insulin resistance and obesity for women participating in the pilot study.

Whilst no lean control group was assessed, the results at 28 weeks' gestation in the control group were similar to those reported for obese pregnant women in previous observational studies, which described an elevation of LDL, total cholesterol,

triglycerides, CRP and Il-6 compared to lean controls (Stewart, Freeman et al. 2007, Meyer, Stewart et al. 2013). To the best of our knowledge only two recently published studies of smaller size (n=27 and 42 respectively and n=58 for UPBEAT controls), have included longitudinal measurement of adiponectin in obese pregnant women (Meyer, Stewart et al. 2013; Ramirez, Miller et al. 2014). In both studies, concentrations of plasma adiponectin were not only comparable to values observed in this thesis but also significantly lower in obese women compared to lean controls (Meyer, Stewart et al. 2013, Ramirez, Miller et al. 2014).

The participants in this thesis therefore share some similarities in terms of biomarkers compared to previously published data in obese pregnant women. A detailed description of results obtained following an overnight fast in the 2nd trimester in the two previous similar studies is given below (Table 35) compared to data collected in this thesis (control subjects only who received standard antenatal care). The most notable differences are observed for plasma insulin and leptin.

Table 35 A comparison of observational longitudinal studies in obese pregnant women, measuring a similar panel of biomarkers to those included in this thesis. Data is reported for the 2nd trimester only and the control arm of the UPBEAT pilot study (Stewart, Freeman et al. 2007, Meyer, Stewart et al. 2013).

	This Thesis*	**Meyer et al***	**Stewart et al****
Number of subjects (n)	58	27	30
BMI (kg/m^2)	35.9 (4.8)	33.7 (4.2)	34.2 (4.5)
Age (years)	30.8 (4.9)	29 (6)	28.7 (1.1)
Total cholesterol (mmol/l)	6.07 (1.21)	5.82 (1.06)	-
LDL (mmol/l)	3.21 (1.38)	3.79 (0.97)	-
Triglycerides (mmol/l)	1.92 (1.48)	2.45 (0.94)	-
Insulin (mU/l)	14.62 (1.99)	58 (41)	-
Leptin	60.37 (1.43)•	44 (20)◆	-
Adiponectin (µg/ml)	5.39 (2.08)	6.3 (2.8)	-
NEFA (mmol/l)	0.31 (0.17)	0.24 (0.34)	-
IL-6 (pg/ml)	1.13 (2.35)	-	1.80 (1.10-3.13)
CRP (mg/l)	8.20 (1.87)	-	8.58 (4.23-12.6)

Data is reported as *mean (SD) and **median (IQR)

Leptin reported in •pg/ml and ◆ng/ml

All blood samples taken following an overnight fast at equivalent gestational age: 27^{+0}-28^{+6} weeks' (UPBEAT), 24-28 weeks' (Meyer et al.) and 2nd trimester (Stewart et al., actual GA not reported)

Despite changes in a 'healthy direction' in the diet as evaluated in the pilot study, it was found that there were no differences in biomarkers between randomised groups on completion of the intervention at 28 weeks'. However by late gestation, women in the intervention arm had significantly lower concentrations of cholesterol, LDL and visfatin. Very few intervention studies in obese pregnant women have previously determined the effect of the intervention on relevant biomarkers, and none as comprehensively as this study in the range of the biomarkers assessed.

A study in 50 obese Caucasian women (mean BMI 34kg/m^2), of a very intensive dietary intervention demonstrated significant improvements in serum insulin, leptin and blood glucose concentrations, compared to the control group who received no dietary support. The intervention of ten 1-hour sessions with a research dietician, was principally designed to limit GWG to 6-7kg, adopting official Danish dietary recommendations (total energy from fat 30%E, protein 15-20%E, carbohydrate 50-55%E), individualised for each subject from 15 weeks' (Wolff, Legarth et al. 2008). The primary outcome was achieved with a 6.7kg difference in GWG observed between the two groups in the absence of any adverse effects on fetal growth or pregnancy outcomes. At 27 weeks' a significant 20% reduction in fasting serum leptin (p=0.004) and insulin (p=0.04) was observed in the intervention arm, with no difference in glucose concentrations following a 50gram OGTT. By late gestation however, this divergence was limited to insulin with a further 23% reduction (p=0.022, leptin p=0.201), accompanied by an 8% reduction in fasting plasma glucose only (p=0.03) with no difference in glucose at 2 hours post OGTT. For the 3 women diagnosed with GDM in the control arm (10%), compared to none in intervention, data was excluded from analysis since standard treatment included dietary advice.

This is in contrast to findings from the LiP study from Denmark the largest comparable RCT in obese pregnant women (n=304, mean BMI 33kg/m^2) which found no difference in the concentration of fasting total cholesterol, HDL, LDL and triglycerides in each trimester, despite achieving significantly lower GWG of -1.6kg in the intervention group (Vinter, Jorgensen et al. 2014). Nonetheless a small improvement in insulin resistance, defined by a significantly smaller change in

HOMA-IR from baseline to 28 weeks' was reported although by late gestation, this was no longer significant.

In comparison to the study by Wolff and colleagues (n=50), LiP (n=304) was a hybrid intervention of 4 individual dietary sessions plus 20 1-hour aerobic exercise sessions with free gym membership (Wolff, Legarth et al. 2008, Vinter, Jensen et al. 2011). Adherence to the dietary component was high at 92% but low for the exercise classes with mean attendance of 10.4 hours out of a maximum of twenty. Populations from both studies were exclusively Caucasian and comparable at baseline (mean age 29 years and BMI 35v33kg/m^2) with fasted samples obtained throughout. This was in contrast to UPBEAT where fasted samples were measured following OGTT at 27-28 weeks'. Both studies were successful in their aim to limit GWG to 6-7kg but a much greater reduction in GWG between study arms was achieved by subjects in the Wolff study (6.7kg v 1.2kg in LiP), yielding a possible explanation for the significant improvements in plasma insulin and leptin concentrations, not observed in LiP. Although the detailed composition of the reduced GWG was not defined to confirm if this was isolated to adipose mass, these results do highlight the potentially important role of adipose tissue as a secretary organ in the pathogenesis of insulin resistance in pregnancy.

Further explanations for the positive findings observed by Wolff et al., might lie within the high intensity study design of 10 compared to 4 dietary consultations, which appear to have greater compliance than physical activity sessions, following review of the data (Luoto, Kinnunen et al. 2011, Oostdam, van Poppel et al. 2012). Consistently reported barriers to greater physical activity in pregnancy include movement restriction associated with advancing gestation and unsupported fears of harm from additional exercise, together with work and childcare commitments. Current evidence suggests that overweight and obese women may benefit the most from diet and exercise programmes in terms of greater reductions in GWG and measures of insulin resistance compared to lean women but translation into acceptable large scale RCTs is lacking (Ong, Guelfi et al. 2009, Nascimento, Surita et al. 2011). Following on from this, it is important to note that unlike the LiP protocol where pre-existing diabetes was excluded at enrolment by OGTT, women in the Wolff study may have had undiagnosed IGT or T2DM. It is therefore plausible

that the degree of insulin resistance was greater overall, providing a greater scope for change (Wolff, Legarth et al. 2008).

The most recent feasibility study of a dietary intervention in obese pregnant women has focused specifically on Hispanic women who form the largest ethnic minority group in the United States (Hawkins, Hosker et al. 2015) (http://www.census.gov/prod/2007pubs/acs-03.pdf). With the highest birth rates and almost 2-fold increased risk of developing GDM compared to White European women of equivalent BMI (Bardenheier, Elixhauser et al. 2013), understanding if targeted interventions are successful in the most at-risk groups is essential. The importance of this is particularly pressing since the risk of T2DM following GDM is greatest in the initial 5 years for all women, during which time almost 50% of Hispanic women will have had a diagnosis confirmed (Kjos, Peters et al. 1995).

In this small study (n=68), women with pre-pregnancy of $\geq 25 kg/m^2$ were randomised to standard care or a 6-month programme of monthly individual consultations supported by 5 telephone sessions, delivered by a healthcare trainer (Hawkins, Hosker et al. 2015). The aims were to achieve American College of Obstetricians and Gynaecologists recommended levels of activity in pregnancy, reduce saturated fat and increase fibre intake via a programme modified to meet the cultural and linguistic needs of this group. Whilst there was no significant effects of the intervention on surrogate measures of insulin resistance or diet at 24-28 weeks' (fasted plasma glucose, insulin, leptin, adiponectin, resistin, TNFα, CRP, total caloric intake, %E fat and fibre consumption), retention in this hard to reach group was high in both arms at 97%. Compared to non-Hispanic White counterparts, the same research group have shown that Hispanic women are less likely to engage in vigorous physical activity in pregnancy (Lynch, Landsbaugh et al. 2012). Therefore and perhaps more importantly, this study demonstrated increased levels of vigorous-intensity activity together with an attenuation of pregnancy associated reductions in moderate physical activity following the intervention.

In terms of modifying biomarkers associated with the development of GDM and subsequent T2DM, in a favourable direction, results from the UPBEAT pilot are promising. Significant reductions not previously reported in comparable studies, were achieved for cholesterol, HDL and visfatin in late pregnancy (34^{+0}-35^{+6}

weeks'), suggesting a late and sustained effect of the intervention not apparent on completion of the intervention at 27^{+0}-28^{+6} weeks'. Biochemical data from the LIMIT study which included overweight and obese women (n=2012 with 56.8% BMI≥30kg/m^2) is awaited but in keeping with published dietary data from the UPBEAT pilot (Poston, Briley et al. 2013), a reduction in percentage dietary intake from saturated fats has been reported, although of a lower magnitude (p=0.04) (Dodd, Cramp et al. 2014). When compared to similar RCT's, the UPBEAT cohort detailed in this thesis, could be considered as "high risk" for GDM. Using the LiP study as an example, our population was slightly older (30.18 v 29 years), more obese (36.0 v 33.4kg/m^2) and heterogeneous with almost a third of women from black ethnic minorities (Vinter, Jensen et al. 2011). Until recently, obese pregnant women participating in lifestyle RCTs have predominantly come from lower-risk Caucasian groups (Wolff, Legarth et al. 2008, Luoto, Kinnunen et al. 2011, Oostdam, van Poppel et al. 2012, Walsh, McGowan et al. 2012, Dodd, Turnbull et al. 2014).

With limited resources to provide lifestyle programmes to all obese women in pregnancy, stratification to identify those at greatest risk of pregnancy related morbidity and heightened lifetime risk of metabolic dysfunction, is required to determine who would benefit most from such approaches. Data for the most successful and acceptable strategies, modified to meet the changing needs of pregnant women are now beginning to emerge for these specific groups.

HDL/LDL Cholesterol

The difference in LDL cholesterol between the intervention and control group at 36 weeks' gestation (p=0.02) may reflect an improvement in cardiovascular and metabolic risk in the trial participants. It might be anticipated that we would have observed a similar fall to that in LDL in the inflammatory mediators measured but none was observed. However there was a non significant reduction in CRP (p=0.08) which will be of interest to assess in the full UPBEAT cohort.

Strong positive associations between total cholesterol and LDL with inflammation, hepatic dysfunction and T2DM have been consistently reported (Sattar, Wannamethee et al. 2008). In early pregnancy obese women present with greater

concentrations of total cholesterol, LDL cholesterol, triglycerides (Ramsay, Ferrell et al. 2002, Scifres, Catov et al. 2014, Farias, Franco-Sena et al. 2015) and CRP (Stewart, Freeman et al. 2007) compared to lean controls. In contrast, explorative analysis of all participants in the UPBEAT pilot to examine for associations between BMI and the panel of biomarkers measured did not yield significant correlations for any measures of lipid metabolism but at baseline, significant associations were found for Il-6, insulin, leptin and CRP (Table 36).

Table 36 Summary of Pearson's correlation analysis for the control and intervention arms combined of BMI against lipid, inflammatory and hormonal biomarkers measured in the UPBEAT pilot study at baseline and post intervention.

	Baseline (15^{+0}-17^{+6} weeks')*		**Post Intervention (27^{+0}-28^{+6} weeks')****	
	Correlation Coefficient (r)	**P Value**	**Correlation Coefficient (r)**	**P Value**
Total cholesterol	-0.11	0.25	-0.09	0.33
Triglycerides	-0.06	0.50	-0.10	0.30
LDL	-0.02	0.81	-0.01	0.84
HDL	-0.18	0.06	-0.15	0.12
VLDL	-0.07	0.49	-0.10	0.30
Cholesterol:HDL Ratio	0.10	0.33	0.07	0.46
LDL:HDL Ratio	0.09	0.34	0.08	0.44
Il-6	0.30	0.002	0.30	0.002
tPA	-	-	-0.24	0.02
Insulin	0.28	0.003	-	-
Leptin	0.41	<0.001	0.35	0.0002
CRP	0.28	0.004	0.26	0.007

Combined data for women in the control and intervention arm is presented (BMI≥30kg/m^2 for all)

Only significant associations are presented for inflammatory and hormonal biomarkers

*Non-fasted samples measured at randomisation

**Fasted samples measured during OGTT

In a smaller study of 55 women (n=27 obese), a significant association between maternal BMI was identified for triglycerides only (p=0.03) but nonetheless, subfraction analysis of LDL cholesterol revealed important differences related to adverse clinical risk for obese women (Meyer, Stewart et al. 2013). A high concentration of small dense LDL-III particles (>50%), termed "Pattern B", is

associated with a pro-atherogenic profile and characterises conditions related to ectopic hepatic fat such as T2DM (Dallmeier and Koenig 2014) and non-alcoholic steatohepatitis (Sugino, Kuboki et al. 2011). Due to the size and density of LDL-III, it has been postulated that invasion of the endothelial wall is facilitated and oxidation occurs more readily with a deleterious effect on vascular wall function particularly in individuals with an increased predisposition for cardiovascular disease (Aragones, Ferre et al. 2012). By late pregnancy (mean 35.5 weeks'), obese women had greater concentrations of LDL-III (p=0.014) and lower concentrations of the intermediate sized LDL-II (p=0.002), equating to 35% displaying a Pattern B profile in contrast to 14% of overweight and 0% of lean women. With just over a third of obese women classified as having Pattern B, the results reaffirm current evidence that only a proportion of obese women exceed the normal metabolic capacity of pregnancy induced hypertriglyceridaemia, accumulate ectopic fat stores, and thus face an increased risk of GDM.

Across the UPBEAT cohort included in this thesis, concentrations of plasma LDL increased in a stepwise manner with advancing gestation but to a lesser degree in the intervention arm. In a prospective observational study of 299 Brazilian women (59.8% lean, 26.2% overweight and 14% [n=32] obese) the rate of change for LDL and triglycerides in obese women was significantly lower compared to lean subjects such that by the end of pregnancy, concentrations were comparable across all groups (Farias, Franco-Sena et al. 2015). Possible explanations for this and similar findings published recently (Meyer, Stewart et al. 2013, Scifres, Catov et al. 2014) may lie in altered gene expression in the adipose tissue of obese women. Using quantitative reverse transcription PCR (qRT-PCR), Lappas et al. demonstrated reduced expression of genes involved in multiple pathways of lipid metabolism including fatty acid and intracellular transport, lipolysis, and triglyceride synthesis in both subcutaneous and omental fat of obese women at term (Lappas 2014). By examining matched controls with normal glucose tolerance and GDM, they were able to extend on their previous work to confirm that insulin resistance in pregnancy is characterised by impaired cellular uptake, synthesis and breakdown of lipid components in the adipose tissue of women with pre-existing obesity and GDM (Colomiere, Permezel et al. 2010).

Visfatin

As the normal physiology of visfatin continues to be mapped out, its possible pathogenic role is less clear (Arner 2006). Greater concentrations of visfatin have been reported in states of insulin resistance (IR) including T2DM and obesity (Fukuhara, Matsuda et al. 2005, Chen, Chung et al. 2006). For GDM, data has been conflicting with both higher (Ferreira, Rezende et al. 2011) and lower concentrations (Haider, Handisurya et al. 2007, Park, Kim et al. 2013) observed when compared to women with normal glucose tolerance (NGT). Almost all published data for GDM has included samples obtained at 24 weeks' onwards, but a significant increase in visfatin, prior to the diagnosis of GDM at 11-13 weeks', was found by Nicolaides et al. (Ferreira, Rezende et al. 2011) In contrast to adiponectin, visfatin concentrations in this study were not significantly affected by maternal age, BMI, smoking status, parity or ethnicity, a finding confirmed more recently by Park et al.(Park, Kim et al. 2013). The relationship of adiponectin with these clinical variables is discussed in detail in 8.2 Prediction of GDM in UPBEAT pilot, page 165.

Visfatin is expressed and secreted predominately by visceral adipose tissue with evidence of a placental origin during pregnancy (Ma, Cheng et al. 2010). The contribution of placental visfatin to systemic maternal concentrations is unclear since increased expression of visfatin has been noted in omental fat of pregnant women compared to non-pregnant controls with little difference in circulating concentrations, suggesting an auto or paracrine function (Briana and Malamitsi-Puchner 2009). It has been described in the literature as an "insulin-mimetic" with "anti-diabetogenic" properties. A series of review articles confirm that visfatin stimulates adipose and muscle uptake of glucose, inhibits hepatic gluconeogenesis, increases phosphorylation of proteins involved in post-receptor insulin signalling and binds to the insulin receptor although most likely to a different location than insulin itself (Fukuhara, Matsuda et al. 2005, Arner 2006, Kim da, Kang et al. 2014). Considering these roles, it has been postulated that high concentrations of visfatin, observed in selected GDM studies, is a consequence of hyperglycaemia and impaired adipokine action, similar to mechanisms of hyperinsulinaemia seen in IR in response to insufficient or impaired insulin secretion.

At present it is unclear whether a reduction in visfatin, achieved following a lifestyle intervention as found in this thesis, has beneficial implications for health. Outwith of pregnancy, current evidence would indicate favourable associations of reduced visfatin concentrations with T2DM risk although the direction and mechanisms involved in this relationship are not fully understood.

Analysis of the UPBEAT study (n=1555) will provide important information as to whether modification of biomarkers associated with metabolic dysfunction and cardiovascular morbidity is achievable during the window of opportunity that pregnancy presents. Women with previous GDM have a 7-fold increased risk of developing T2DM (Bellamy, Casas et al. 2009) therefore any intervention that may interrupt the cycle perpetuated by obesity to reduce or delay the onset of T2DM beyond pregnancy would be important.

8.2 Prediction of GDM in UPBEAT pilot

The second study in this thesis addressed the potential for the use of a combination of biomarkers and clinical factors in the prediction of gestational diabetes in the UPBEAT pilot cohort.

Research into the prediction of adverse outcomes in other pregnancy related conditions such as pre-eclampsia has shown that a combination of clinical history and early pregnancy clinical measures, together with addition of biomarkers measured in biological samples may provide an effective strategy in early pregnancy risk assessment (Akolekar, Syngelaki et al. 2013). Several studies have adopted this approach in prediction of GDM (Ferreira, Rezende et al. 2011, Lacroix, Battista et al. 2013), but to our knowledge, not previously in a population of obese women.

This study highlights novel biochemical and clinical factors for the prediction of GDM in obese pregnant women and suggests that an algorithm based on simple clinical variables plus adiponectin may provide a clinically useful method for prediction of GDM in this population.

Four previous studies have identified a number of patient characteristics and biomarkers associated with the prediction of GDM in cohorts of mixed BMI (Savvidou, Nelson et al. 2010, Ferreira, Rezende et al. 2011, Nanda, Savvidou et al.

2011, Gobl, Bozkurt et al. 2012). These have been undertaken in populations of mixed risk, including non-Caucasian ethnicity (Ferreira, Rezende et al. 2011, Nanda, Savvidou et al. 2011, Gobl, Bozkurt et al. 2012), a family history of diabetes (Savvidou, Nelson et al. 2010, Ferreira, Rezende et al. 2011, Nanda, Savvidou et al. 2011, Gobl, Bozkurt et al. 2012), previous history of GDM (Ferreira, Rezende et al. 2011, Nanda, Savvidou et al. 2011, Gobl, Bozkurt et al. 2012), increased pre-pregnancy BMI (Savvidou, Nelson et al. 2010, Ferreira, Rezende et al. 2011, Nanda, Savvidou et al. 2011), increased maternal age (Ferreira, Rezende et al. 2011, Nanda, Savvidou et al. 2011, Gobl, Bozkurt et al. 2012) and of differing parity (Savvidou, Nelson et al. 2010).

Savvidou et al. measured nine biomarkers in the first trimester and found that high tPA and low HDL increased the AUC-ROC from 0.824 with clinical risk factors alone to 0.861 in a group of all comers regardless of baseline BMI (Savvidou, Nelson et al. 2010). The addition of adiponectin to prediction models for GDM has consistently increased the AUC-ROC to values above those achieved with clinical measures alone. Further inclusion of adipokines and biomarkers has frequently demonstrated a modest, non-significant increase in the AUC-ROC. For example, in a case controlled study of 400 women, those with GDM were reported to have increased maternal serum visfatin and decreased serum adiponectin concentrations at 11-13 weeks. The addition of adiponectin to the prediction model using clinical measures alone resulted in a significant change in the AUC-ROC whereas there was a non-significant increase with addition of visfatin (AUC-ROC 0.828 [maternal characteristics alone], 0.854 [adiponectin] and 0.855 [adiponectin and visfatin]) (Ferreira, Rezende et al. 2011). Nanda et al. measured three biomarkers and found that in the GDM group, compared to controls, adiponectin and sex hormone-binding globulin (SHBG) were lower. When screening for GDM by maternal characteristics alone, the detection rate was 61.6% (false-positive rate of 20%) increasing to 74.1% with the addition of adiponectin and SHBG (Nanda, Savvidou et al. 2011).

Alternative approaches to GDM risk assessment have included measurement of biomarkers in the preconception period, a recent report finding that maternal characteristics, fasting plasma glucose, glycosuria and preconception dyslipidaemia yielded an AUC-ROC of 0.90 for the prediction of GDM (Gobl, Bozkurt et al.

2012). However, the various diagnostic criteria for GDM used in previous studies have limited comparisons between previous attempts to predict GDM. Importantly, none has specifically addressed risk assessment in obese pregnant women, which has important implications for clinical practice given the recognition of obesity as the major risk factor for GDM, and the likelihood that the biomarker profile in women with a high BMI may be dissimilar from other risk groups.

Our results suggest that clinically useful prediction of GDM in obese pregnant women may be achievable using a combination of clinical characteristics (older age, increased blood pressure [SBP and DBP], parity ≥2 and black ethnicity) combined with the plasma concentration of adiponectin but more data is required from larger adequately powered studies prior to recommended use in clinical practice; this will be expanded in 8.2.1 Limitations, page 170. To reflect current clinical practice, routine clinical measurements recorded at antenatal visits were included. The inclusion of detailed maternal anthropometry (including skin-fold thicknesses), which is undertaken in all women participating in the UPBEAT trial, suggested a limited potential role for taking such measurements routinely as an aid to GDM prediction. Positive linear associations between upper arm skinfold thickness and maternal glucose concentration at 24-28 weeks' have been reported by Tomedi et al. in a sub-sample of 214 women participating in the Study of Nutrition and Pregnancy Trial (mean BMI 28kg/m^2) (Tomedi, Simhan et al. 2014). For every standard deviation increase in arm skinfold thickness (8.8mm biceps and 11.7mm triceps), a 4.3mg/dL increase in maternal glucose (0.2mmol/l) was observed. Importantly, results were adjusted for established clinical confounders (pre-pregnancy BMI ethnicity, age, parity, education level and family history of diabetes) and measurements recorded at one time period only <13 weeks' gestation when fat accretion is minimal and hence most likely to reflect true pre-pregnancy anthropometry. Practical barriers to greater uptake in clinical medicine include the additional time required for triplicate measures and the requirement of adequate training for staff.

Adiponectin, an adipocyte derived adipokine, is now recognised as being strongly associated with improved glucose metabolism although the causality of this relationship remains debated. Irrespective of causal direction, adiponectin appears to

provide a good 'read-out' of whole body insulin sensitivity. In a recent meta-analysis of non-pregnant individuals adiponectin was shown to be strongly predictive of type 2 diabetes, and inversely related to measures of insulin resistance and BMI. In this study, we have shown similar values for adiponectin at 28weeks' gestation (5.39μg/ml [2.08]) to a previous report for a small cohort of obese women studied in Glasgow, which showed lower values than lean controls and a strong association between adiponectin and BMI (p=0.002) (Meyer, Stewart et al. 2013).

The role of adiponectin in obese pregnant women may extend beyond usefulness as a biomarker. In the Hyperglycemia and Adverse Pregnancy Outcome (HAPO), serum concentrations of adiponectin declined as glucose and maternal BMI increased and adiponectin was inversely associated with birth weight, neonatal skin fold thickness and total body fat (estimated using anthropometry), giving rise to the hypothesis that this cytokine may play a role in fetal growth regulation by modulation of placental nutrient transport in addition to maternal glucose homeostasis (Lowe, Metzger et al. 2010). Data in support of a placental origin of adiponectin remains equivocal, with evidence favouring maternal origin of adiponectin measured in the blood of pregnant women (Aye, Powell et al. 2013). Maternal adiponectin has, therefore, the potential to be a 'functional' target for interventions in obese pregnant women whereby achievement of increased plasma concentrations could parallel a reduced risk of macrosomia, although in the UPBEAT pilot study we found no significant increase in the intervention arm. However, adiponectin has been shown to be modifiable by dietary intervention in non-pregnant populations (Esposito, Pontillo et al. 2003). Lifestyle interventions in pregnant women of differing pre-pregnancy BMI categories have been equivocal in regard to effects on glucose metabolism and insulin resistance although none has previously measured adiponectin (Barakat, Cordero et al. 2012, Oostdam, van Poppel et al. 2012). On completion of UPBEAT (n=1555), the influence of the intervention on plasma adiponectin concentration in the whole cohort will therefore be explored.

To the best of our knowledge there is only one other recently published study of adiponectin and GDM in an exclusively obese population (Ramirez, Miller et al. 2014). Comparisons of findings with those in this thesis are limited due to numerous differences in protocol design despite similar outcomes for adiponectin. Ramirez et

al. recruited almost exclusively Hispanic women (92%) women at 24-28 weeks' gestation, with maternal blood sampled at one time point only. Within 2 weeks of enrolment, 30 cases of GDM were confirmed using the 2-step approach of 50gram glucose challenge followed by 100gram OGTT instead of the IADPSG recommendations. Although the total sample was smaller than the UPBEAT pilot, the incidence of GDM was higher (44.4% v 27.4%) as potentially anticipated in this ethnic group. In contrast, generation of the UPBEAT prediction model, incorporated adiponectin values predating the diagnosis of GDM at 15^{+0}-17^{+6} weeks' which is likely to carry greater relevance when translated into routine clinical care.

The UPBEAT pilot findings, regarding adiponectin, are consistent with other reports in women of all BMI categories prior to the development of GDM or with established disease (Retnakaran, Hanley et al. 2004, Nanda, Savvidou et al. 2011). A Brazilian case controlled study, confirmed significantly lower concentrations of serum adiponectin in women with GDM, independent of BMI, in the third trimester (28-36 weeks') ($p=0.0015$). Entry to the study was unrestricted by pre-pregnancy BMI however in keeping with published data, women with GDM were older with greater pre-pregnancy BMI (28.9 v 23.2kg/m^2, $p<0.001$) (Gueuvoghlanian-Silva, Torloni et al. 2012). In contrast, although adiponectin was significantly lower in women who developed GDM in a previous study in women of mixed risk, it did not contribute to the final model which combined two factors (HDL-c and t-PA antigen), both recognised to be related to adiponectin via linked hepatic/circulating triglyceride-mediated pathways (Sattar, Wannamethee et al. 2008).

Low serum adiponectin concentrations appear to be associated with ethnic groups known to have a higher risk of developing incident T2DM later in life (Lindsay, Funahashi et al. 2002). In the present study, women of black ethnic origin had significantly lower plasma levels of adiponectin than non-black women, in keeping with findings from previous work examining pregnant women of South Asian origin (Retnakaran, Hanley et al. 2004). Whilst we acknowledge our diverse ethnic population is not representative of the UK, having a large proportion of women of black ethnic origin (>80%) has yielded further insights into the relationship between adiponectin and ethnicity.

We also observed a strong association between concentration of adiponectin and smoking status with a 50% reduction observed in smokers ($p<0.001$). Similar findings have previously been reported in a non-pregnant population in which the plasma adiponectin concentration increased in a stepwise fashion with "never, past and current smokers" (Miyazaki, Shimada et al. 2003).

To date, current studies of dietary/ physical (Han, Middleton et al. 2012, Poston, Bell et al. 2015) and pharmacological (Chiswick, Reynolds et al. 2015) interventions have failed to demonstrate prevention of GDM in overweight or obese women. Nonetheless early prediction of GDM in this high-risk group has potential clinical implications. Positive maternal outcomes in terms of limited GWG reduction, increased physical activity and dietary modifications have been reported (Dodd, Cramp et al. 2014, Poston, Bell et al. 2015), with such findings forming part of an important overarching public health strategy. Whilst obstetric and neonatal outcomes are unchanged including the incidence of LGA, the LIMIT study reported a significant relative risk reduction of 18% in infant birth weight >4kg (Dodd 2014). Until improvements to defined clinical outcomes are demonstrated, the provision of lifestyle intervention programmes to all overweight and obese women is financially restricted. However since obese women remain at risk of adverse obstetric and long-term metabolic sequelae, identifying those at greatest risk within this population would seem a reasonable approach.

8.2.1 Limitations

Fasting blood samples were not obtained at randomisation (15^{+0}-17^{+6} weeks'), precluding the measurement of the fasting glucose or insulin concentration and thus assessment of HOMA-IR. However, as fasting is not mandatory for antenatal clinic visits, this study was designed pragmatically, to be relevant to current clinical practice in obese women.

The most notable limitation of the prediction model was the sample size for the number of predictors considered (GDM n=28 and non-GDM n=77). Extended reading in this field has raised multiple important principles detailed in the Transparent reporting of a multivariate prediction model for Individual Prognosis or Diagnosis (TRIPOD) statement (Collins, Reitsma et al. 2015). For logistic regression

analysis as used in prediction models or risk scores, statistical guidance recommends a minimum of 10 cases per independent variable (Hosmer, Lemeshow et al. 2013), a figure extended up to 30 by Steyerberg et al. (Steyerberg, Vickers et al. 2010). More than ten clinical variables were included in preliminary univariate analysis, 7 of which were found to be significant (age parity ≥2, black ethnicity, SBP, DBP, triceps skinfold and sum of skinfolds). A total of 18 biomarkers were included in the analysis and following adjustment for the main clinical predictors (with the exception of anthropometry) only adiponectin was predictive of GDM.

Applying these key principles, for the clinical predictors alone (n>10), 100 cases of GDM are required in contrast to the 28 cases in this study. Inclusion of all clinical and biochemical variables (n>30) would require 300 cases of GDM and a population sample size of over 900 subjects. This was an exploratory project using data from the UPBEAT pilot study with an inappropriately small sample size therefore the results are to interpreted with caution. Replication of this prediction model or development of a new one on the now complete UPBEAT cohort (n=1555, GDM cases=172) would need to take the recently published guidance into consideration.

Internal validation of the model using discrimination with ROC curve analysis was performed. Other methods of internal validation including calibration were not used and external validation was outwith the scope this thesis. The initial aims was to utilise the completed UPBEAT cohort for the purpose of external validation but following review of the TRIPOD statement, revaluation together with redevelopment of this prediction model is advisable (Collins, Reitsma et al. 2015).

The sample size calculation was for outcomes defined for the UPBEAT study and not specifically for those measured in this thesis. Data on similar studies in obese pregnant women was unavailable for power calculations in the preliminary stages of the protocol development for UPBEAT. The pilot study was powered for a change in dietary and PA behaviours at 28 weeks' when the OGTT was performed. Phase 2 of UPBEAT, known as the "exploratory stage" was set within predefined time limits and the pilot size of 183 subjects was derived from this. This was an adequate number to allow for power calculations for the primary endpoints of GDM and LGA in the main RCT with an estimated variance of the true value of 7%.

In this high risk group, it is possible that some women with GDM may have had undiagnosed T2DM since initial screening with FBG or HbA1c was not undertaken, as IADPSG recommendations for universal testing for overt diabetes at the first antenatal visit have not been implemented. Thus, it is possible that low early pregnancy adiponectin concentrations may have been influenced by T2DM in a small minority of women. However others have shown that low adiponectin concentrations are strongly predictive of GDM in women in whom T2DM was excluded antenatally (Hedderson, Darbinian et al. 2013).

The algorithm developed in this study was based on the diagnosis of GDM by IADSPG criteria; it follows that it is potentially valid only for this method of diagnosis. Whilst IADPSG is increasingly being adopted, e.g. by the WHO, it would be appropriate to evaluate the predictive potential of the model in larger studies adopting other commonly used criteria.

8.3 Summary

In summary, we have made the novel observation that the risk of developing GDM in obese pregnant women may be predicted in the early second trimester of pregnancy by using an algorithm which incorporates routine clinical variables as well as the biochemical marker adiponectin. Our findings therefore extend prior studies and collectively suggest that by additionally measuring adiponectin in high-risk women before routine clinical diagnosis of GDM, a potential therapeutic window for intervention could be created. Since GDM is associated with increased risk of incident type 2 diabetes and 10 year cardiovascular risk in mothers (Fraser, Nelson et al. 2012), as well as maternal and neonatal pregnancy complications, successful intervention has the potential to improve both short and long term outcomes. We conclude that further large scale studies of GDM prediction in obese pregnant women are warranted. This study highlights novel biochemical and clinical factors for the prediction of GDM in obese pregnant women and suggests that an algorithm based on simple clinical variables plus adiponectin may provide a clinically useful method for prediction of GDM in this population, and thereby identification of those women who might benefit most from an intervention. Whilst more accurate risk assessment may be costly, the recent demonstration of cost effectiveness using the IADPSG criteria for GDM diagnosis, suggests that initial

added costs may be offset by later gain. Earlier studies adopted the 1990 WHO criteria for GDM and until now, prospective evaluation of the IADPSG guidelines on pregnancy outcomes with cost effectiveness analysis has been unknown. In the first large study of its kind, the St. Carlos Gestational Diabetes Study reported significant reductions in multiple adverse clinical outcomes, coupled with estimated financial savings of €14, 000 per 100 women evaluated despite the increase in GDM rate (35.5 v 10.6%) when comparing 2 cohorts of women diagnosed by IADPSG and the 2-step 100 gram OGTT, currently recommended by the American College of Obstetricians and Gynaecologists (Duran, Saenz et al. 2014).

The next step: offspring outcomes

Offspring of obese women and those with GDM face an increased lifetime risk of abnormal metabolic outcomes associated with morbidity, inferring a role of intrauterine hyperglycaemia or early life exposures on the developing infant (Clausen, Mathiesen et al. 2008). Animal models have demonstrated multiple sequelae of maternal hyperglycaemia including impaired insulin signalling and pancreatic β-cell function, abnormal hypothalamic development associated with energy dysregulation and altered fat distribution in pups (Poston 2010). It is hypothesised that modification of the intrauterine environment to reduce fetal exposure to hyperglycaemia may influence maternal programming however the long term effects have not been addressed in large prospective RCTs other than a recent follow up study from the LiP cohort discussed in 1.5, page 58. Body composition measured by DEXA, anthropometry and surrogate markers of metabolic function (insulin, lipids, glucose and blood pressure) were recorded in 157 children up the age of 2.8 years in The Lifestyle in Pregnancy and Offspring (LiPO) follow-on study. No detectable effect of the intervention was found between the two arms of children participating either in the main trial or between the reference group of 97 children born to women of normal BMI (Tanvig, Vinter et al. 2014, Tanvig, Vinter et al. 2014). Retention of participants for longitudinal data is notoriously difficult and at present the LiPO is the largest published cohort from an exclusively obese sample. Whether positive findings of such interventions translate into clinically meaningful offspring effects, remains unanswered but collaborative working from the LIMIT

(Dodd, Turnbull et al. 2014), ROLO (Walsh, Mahony et al. 2014) and UPBEAT (Briley, Barr et al. 2014) groups will generate important answers.

9 DISCUSSION: IGPOP, A PILOT STUDY OF AN LGI DIETARY INTERVENTION

The third study in this thesis (IGPOP) carried out necessary preliminary investigations to inform a clinical trial of an LGI slow digesting carbohydrate supplement in obese pregnant women.

Evidence to support LGI diets in pregnancy has not been consistent or reproducible but has yielded important information regarding safety. It should be noted however, with exception of the LIMIT study (inclusion BMI≥25kg^2), that none of the trials thus far have been designed specifically for obese pregnant (OP) women (Louie, Markovic et al. 2011, Marathe, Rayner et al. 2013, Moses, Casey et al. 2014, Walsh, Mahony et al. 2014).

In the main, pregnancy outcomes in LGI dietary RCTs are unchanged but a review of the data does suggest several benefits with regards to reduction in GWG (Rhodes, Pawlak et al. 2010, Walsh, McGowan et al. 2012), positive associations with maternal HbA1c and plasma glucose concentrations (Scholl, Chen et al. 2004, Walsh, McGowan et al. 2012) and a reduction in progression to insulin therapy in GDM, coupled with reduced healthcare costs (Moses, Barker et al. 2009). Additionally, for women with GDM, diets with high glycaemic load (GL) are associated with an overall reduction in quality reflected by lower intakes of monounsaturated and polyunsaturated fats together with vitamin E and potassium ($p<0.001$) (Louie, Markovic et al. 2013).

The most recent systematic review and meta-analysis of dietary interventions in GDM identified 9 eligible RCTs (n=884), categorised by intervention type as follows: LGI (n=4), low CHO (n=2), total caloric restriction (n=2) and others (n=1) based on ethnic variations of diet (Viana, Gross et al. 2014). With a reduction in mean birth weight and treatment with insulin the authors recommended LGI diets as the optimal dietary approach.

As rates of maternal obesity, IGT, and GDM rise at an alarming rate, the need to identify the most effective low-cost intervention cannot be underestimated.

We tested two nutritional drink supplements of identical macronutrient composition in obese pregnant women (BMI 37kg/m^2) without GDM, as part of a calorie controlled diet (CHO 60.7%, fat 20.8% and protein 18.5%). The supplements differed only by nature of CHO, generating a low GI value of 27 for the intervention. In contrast to the control, composed of 100% rapidly digesting CHO, the intervention supplement contained slow digesting CHO (68%), resistant starch (15.5%) and fibre (3.5g). As a consequence, the %E from fat did not increase.

Using CGMS, we demonstrated that consumption of a slow digesting low glycaemic index (SD-LGI) nutritional supplement, specifically developed for use in pregnancy, significantly reduced glucose concentrations over a 24 hour period in addition to day and night periods when examined separately, compared to habitual living ($p<0.001$ for all).

Women were not required to record meal times on the CGMS system during the habitual phase of the study (Saturday and Sunday) therefore comparisons of post prandial glucose concentration (PPG) were only available for controlled study days between the intervention and control. PPG was significantly lower following the intervention for breakfast only ($p=0.03$, 0.34 and 0.71 for breakfast, lunch and dinner). In clinical practice, this is often the most challenging time to maintain BG levels within accepted ranges for women with diabetes in pregnancy due to the normal physiological secretion of insulin counter-regulatory hormones together with high concentrations of processed CHO contained in breakfast foods (Porcellati, Lucidi et al. 2013). This often results in larger doses of insulin being used, associated with a greater risk of hypoglycaemia or alternatively removing CHO altogether, a difficult option for many women. Therapeutic options using resistant or LGI CHO to reduce morning postprandial hyperglycaemia may have the potential for important clinical translation and wide usage not only in GDM but all types of diabetes in pregnancy, owing to the limitations of current agents (Porcellati, Lucidi et al. 2013).

Numerous factors including meal composition, pre-meal glucose concentration, activity levels, insulin secretion, gastric emptying and hepatic glucose metabolism determine post prandial glucose. Hence, the reduction in post prandial glucose observed following traditional CHO restriction, can be explained only in part by the lower total CHO load. Since the rate of gastric emptying is delayed by fat, the

observed increase in %E from fat to approximately 45% following traditional dietary strategies recommended by the American College of Obstetricians and Gynaecologists (2013), will undoubtedly influence PPG, although the relative contribution of these two factors remains unquantified (Marathe, Rayner et al. 2013). In this thesis, improvements in post prandial glucose concentrations were observed without a *reduction in CHO load* or *increase in %E from fat*, excluding this mechanism as a confounder and thus supporting the role of CHO modification.

In this study we were able to demonstrate improvements in post prandial glucose, without the adverse consequences of greater dietary fat, by using complex CHO. Importantly in this obese population, the addition of the nutritional supplement to the controlled diet did not exceed recommended daily energy requirements for the gestational age, (24hr total calorie content 2014kcal with 303kcal contribution from the supplement) (Rasmussen and Yaktine 2009).

Obese women remain at greatest risk of lipotoxicity and its metabolic sequelae. This occurs as a consequence of increased hydrolysis of maternal triglycerides (TGs) from dietary sources and expanded adipose depots generating FFAs, which contribute to IR, cross the placenta and enter fetal adipocytes (Jarvie, Hauguel-de-Mouzon et al. 2010, Catalano and Hauguel-De Mouzon 2011). Maternal concentrations of FFAs and TGs correlate positively with cord blood measures indicating an altered intrauterine environment, shown to be strongly predictive of accelerated fetal growth (Schaefer-Graf, Graf et al. 2008).

It is possible that LGI diets are more effective in obese compared to lean women, where the degree of insulin resistance and beta cell dysfunction/depletion is exaggerated, in terms of improved pregnancy outcomes and overall glycaemia, (Langer, Yogev et al. 2005, Catalano 2007). The majority of adequately powered studies comparing LGI diets in women with GDM (Louie, Markovic et al. 2011, Louie, Markovic et al. 2013, Moreno-Castilla, Hernandez et al. 2013, Moses, Casey et al. 2014) and without GDM, (Walsh, McGowan et al. 2012, Moses, Casey et al. 2014) have reported mean BMIs of 24-27kg/m^2. In the heterogeneous LIMIT population (n=2212), 42% of women were classified as overweight and 58% obese (Dodd, Turnbull et al. 2014). Unlike LIMIT where controls received no dietary advice, most RCTs including those outwith of GDM, have included standard healthy

eating dietary advice for all participants, thereby reducing the effect of any intervention. An early study by Moses et al., reported improved obstetric outcomes (birth weight, ponderal index and incidence of LGA) comparing LGI to a "high-fibre moderate-to-high GI (HGI)" diet (n=62, mean BMI 25.5kg/m^2, mean GI 51 v 58 for LGI and HGI respectively) however this result was not replicated in the larger RCT (mean BMI 24.5kg/m^2) from the same group where the control group received healthy eating advice (Moses, Luebcke et al. 2006, Moses, Casey et al. 2014). In both studies the intervention arm achieved a significantly lower GI than the control but in the former, associated with improved clinical outcomes, a greater point difference in GI was found (7 versus 3) (Moses, Luebcke et al. 2006).

These factors may in part explain the lack of consistent positive findings for studies of LGI diets in pregnancy. Additional confounders include different methods of diagnosing GDM, the optimal target glucose range before introduction of pharmacotherapy and the ethnicity of subjects which would influence not only genetic susceptibility to GDM but dietary habits also.

Considering the potential therapeutic benefits of LGI diets in pregnancy, improving glycaemic control using this approach in high-risk obese women to limit progression to GDM is an area that warrants further exploration. As obese women do not routinely receive dietary advice in pregnancy, future research should include comparison of LGI diets against habitual intake and activity. Participation in any such study would inevitably introduce a degree of bias with control subjects more likely to improve their eating behaviours but as found in this small pilot study, the greatest reductions in glucose concentration were observed between the intervention and habitual diets at all time periods; 24 hour, day-time, night-time and FBG ($p>0.001$ for all). In contrast, smaller yet significant reductions in glucose concentrations were recorded between the intervention and control at 2 time periods; 24 hour and day-time ($p<0.001$ for both).

Glucose concentrations were generally lower on the 1st of each 2 day test period for the intervention and control. Visits on these days were conducted in the clinical research facility (CRF), a highly controlled environment, with limited ability to exercise. This could indicate issues with non-adherence to the prescribed diet on "home" days or more likely reflect a chance finding. Several methods were used to

improve compliance in the IGPOP study. Women were requested to return all empty food packets and drink cartons and complete a food and exercise diary. This was reviewed together with the dietician at each CRF visit. Open contact with the study team via a dedicated mobile telephone number was also available for additional support.

Hernandez et al. conducted a study similar to IGPOP, evaluating the effect of a complex CHO-lower fat (HCC-LF) diet without nutritional supplements in overweight and obese pregnant women (mean BMI 34kg/m^2), using CGMS in controlled and free living environments (Hernandez, Van Pelt et al. 2014). In this small, randomised cross-over study (n=16), subjects had GDM diagnosed by Carpenter and Coustan criteria (FBG 5.3mmol/l, 1 hour 10.0mmol/l, 2 hour 8.6mmol/l or 3 hour 7.8mmol/l following 100 gram OGTT) therefore the comparator diet followed current practice of CHO restriction (CHO 40%, fat 45% and protein 15%). No data for habitual diet was given. Using mean glucose concentrations at various time points, no difference between diets was found but glucose AUC was significantly greater for day and 24 hour periods ($p=0.03$ and 0.02 respectively) and post prandial glucose at 1 and 2 hours ($p\leq0.01$ and 0.001) following the intervention HCC-LF diet. Nonetheless glucose values did not exceed current international treatment targets for GDM (1 hour <7.8mmol/l and 2 hour <6.7mmol/l) (Metzger, Buchanan et al. 2007) and the clinical relevance of this modest increase in glucose exposure on pregnancy outcomes requires further assessment.

In the same study, the AUC for plasma insulin and C-peptide was greater ($p\leq0.001$) and unchanged for TGs following the HCC-LF intervention diet (Hernandez, Van Pelt et al. 2014). For FFAs, a significant 19% reduction was observed. In comparison, we found no difference in plasma concentrations of insulin, C-peptide and TGs following the SD-LGI intervention supplement but a small increase in NEFA ($p=0.049$) following linear regression analysis. This may reflect differences between maternal obesity in the context of normal glucose tolerance and GDM. The trend in NEFA/FFAs was similar across both studies reaching a nadir at 120minutes. Comparisons of biochemistry data from the 2 studies is summarised below (Figure 51).

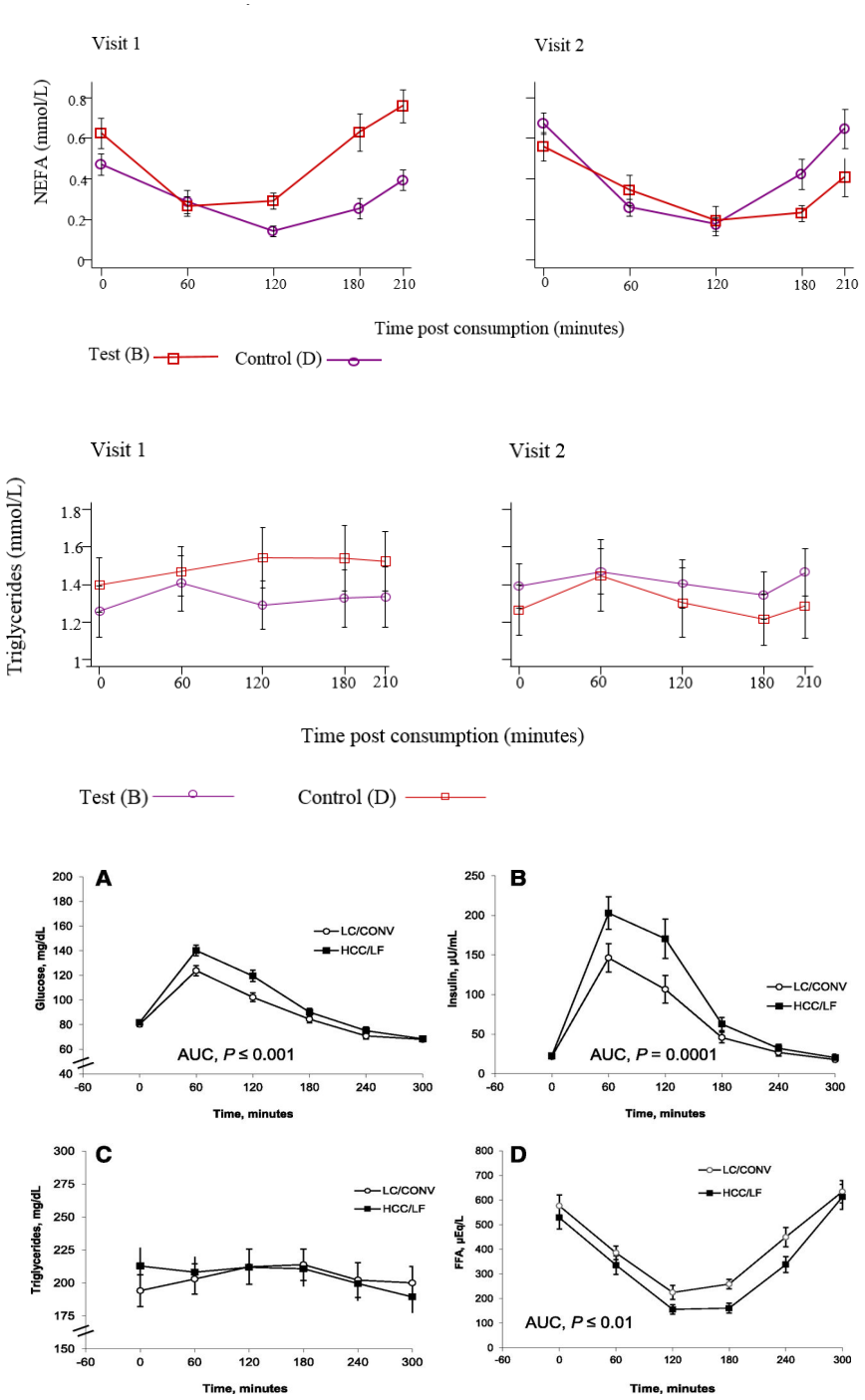


Figure 51 Comparison of biochemistry data for NEFA and triglycerides from the IGPOP study following consumption of a SD-LGI supplement drink in obese pregnant women and following a high-complex CHO low fat diet in women with GDM participating in a study by Hernandez et al. (Hernandez, Van Pelt et al. 2014). Data presented as mean±SEM.

Both populations were obese and ethnically diverse but it should be noted that women in the Hernandez study had GDM and were of greater gestational age (29-32 versus 24-28 weeks'). By definition, when considering these two factors together,

women in their study were more likely to have a greater degree of IR. Results differed by study day in IGPOP despite equivalent controlled conditions for both visits to the CRF. This is likely to be a chance finding as the 2 day washout period was deemed adequate when compared to similar projects and preparation was the same for each visit (Hernandez, Van Pelt et al. 2014). In both studies sample size was small (n=16) and IGPOP was powered only for change in glucose concentrations and not maternal biochemistry. Following review of the available data, with particular reference to the reduction in NEFA achieved in the Hernandez study and similar trends in the IGPOP study, validation of these findings in a larger population may yield important results as to how an obesogenic environment could be modified.

9.1 Limitations

9.1.1 Study design

Randomisation

The simple randomisation software incorporated in the database design did not take into account the order of the drink consumed; sequence 1=intervention/control and sequence 2=control/intervention. Subsequently, the allocation sequence for the initial 22 women was unbalanced with 13 randomised to sequence 1 and 9 to sequence 2. In primary analysis, this was shown to have introduced substantial bias in the CGMS data (Schulz and Grimes 2002).

From a statistical perspective, simple randomisation is not adequate for this type of crossover study design and is more appropriate for sample sizes >200 subjects, since it carries a greater probability of group imbalance, as observed with IGPOP (Lachin, Matts et al. 1988, Schulz and Grimes 2002). In this situation, results can be affected by participant characteristics amongst other factors. Therefore in order to correct for this and minimise bias, a stratified random subsample of 8 patients by sequence, equally weighted by ethnicity was selected.

Additionally, inherent limitations of the FreeStyle® Navigator system, with regards to sensor stability and accuracy, are likely to have influenced preliminary analysis of

the full unbalanced cohort. The first generation Navigator required an initial period of 10 hours before glucose values were reported due to post-insertion variability of sensor function (3-10 hours) caused by local skin trauma and healing (Weinstein, Schwartz et al. 2007). At this time, sensor signal was noted to be reduced leading to inaccuracies. To correct for this, 2nd generation software incorporated an algorithm named TRUstart™, requiring 5 SMBG calibrations to minimise the probability of unstable signal (Geoffrey, Brazg et al. 2011). This 2nd generation system was used in the IGPOP study with CGMS data obtained only after a successful 1hour calibration, following manufacturer's recommendations. Despite this corrective software however, issues of sensor instability are considered to be negligible only after 24 hours. To discard the 1st day of CGMS data in this small proof of concept study would have resulted in 50% data loss. The addition of an extra day prior to each crossover phase, to generate 24 hours of CGMS data and in turn minimize sensor instability, would have impacted recruitment particularly for women in employment or with young children.

Since completion of this study, the next generation Navigator II with improved accuracy has been licensed for clinical use (https://abbottdiabetescare.co.uk/our-products/other-meters/freestyle-navigator-2) and alternative methods of measuring ISF glucose have been launched. The Abbott FreeStyle® Libre uses latest technology to record glucose concentrations in a non-continuous manner using similar enzymatic subcutaneous sensor technology with readings generated only when the user swipes a reader across the sensor (http://www.freestylelibre.co.uk/).

To obviate such issues in a larger RCT, an appropriate randomisation process would be adopted and most up-to-date sensors used.

Due to study design, only CGMS data from the 1st night could be included for each 2 day test period (Thursday–Friday and Monday-Tuesday) since the prescribed diet technically ended on midnight of the 2nd day, leaving the remainder of the night uncontrolled for. Nocturnal results are therefore underpowered but remain promising for a treatment effect of the SD-LGI supplement with lower glucose estimates for the intervention compared to the control and habitual diets ($p=0.09$ and $p<0.001$ respectively). Clear instructions to avoid any oral intake overnight would have

allowed for inclusion of the 2nd night but from review of the data it was clear that individual subjects had eaten from midnight onwards.

Women with confirmed GDM were ineligible for the study. Screening for GDM with a 75 gram OGTT, which would have excluded undiagnosed GDM or T2DM was not performed prior to entry. For one participant, a diagnosis of T2DM was highly likely and she was referred for appropriate medical follow up.

In keeping with the local demography of Guys and St Thomas' NHS Foundation Trust, we had a high number of women from Black ethnic minorities (15/22 and 12/16) who have a significantly greater risk of GDM and T2DM compared to White European women of equivalent BMI (Ferrara 2007). The large reductions in glucose concentrations observed on the intervention diet compared to habitual living may not therefore be as pronounced in a Caucasian population, due to cultural differences in diet. Conversely the results indicate that those at greatest risk of GDM may stand to gain the greatest benefit from such dietary interventions in pregnancy as suggested by Louie et al. (Louie, Markovic et al. 2011).

Habitual data was only collected at the weekend when eating and activity behaviours often differ from during the week when lifestyle is more regulated. This is an important point to consider when drawing conclusions from the data and in the design of future studies.

9.1.2 CGMS technology

A summary of other relevant limitations limited not only to the FreeStyle® Navigator but all commercially available systems is given below:

1. Sensor drop out with loss of data occurs at extremes of temperature, when connection is lost with the receiver unit and in situations such a sleeping when additional external pressures are applied involuntarily. Although there is no clinical restriction to wearing the sensor on the abdomen in pregnancy, all women elected to use the upper arm thereby increasing the amount of nocturnal drop out whilst sleeping in a lateral position.
2. Some women experienced local skin irritation and problems with the sensor falling out particularly in the summer heat when the adhesive did not perform

optimally. In this instance, alternative adhesives were tried and the sensor relocated.

3. The FreeStyle® Navigator only permits the mandatory calibrations when BG>3.3mmol/l. In this non-diabetic population, many subjects experienced BG<3.3mmol/l resulting in a "failed calibration" with interruption to glucose recordings until BG reached the desired range when a calibration was permitted.

9.2 Summary

Using CGMS, we demonstrated that consumption of a slow digesting low glycaemic index (SD-LGI) nutritional supplement, specifically developed for use in pregnancy, significantly reduced the plasma glucose concentration over a 24 hour period in addition to day and night periods when examined separately, compared to habitual living ($p<0.001$ for all) in obese women with no history of GDM in the index pregnancy. Postprandial glucose was lower if the test supplement was given with breakfast, but no different if given with other meals ($p=0.03$, 0.34 and 0.71 for breakfast, lunch and dinner).

This approach to dietary management of hyperglycaemia in pregnancy may be specifically pertinent for obese women to reduce the increased energy contribution from greater fat consumption typically observed with current practice to restrict CHO.

9.3 Future research following on from IGPOP

IGPOP was a proof of concept study to investigate the effects of the SD-LGI supplement and inform the design of the large RCT "NIGO Health- Nutritional Intervention During Gestation and Offspring Health" which has now commenced (ISRCTN: NCT02285764). NIGO Health is part of EarlyNutrition, a research project funded by the European Union, to investigate the effect of early nutrition and lifestyle on metabolic programming (www.project- earlynutrition.eu).

The research programme is centered on 5 scientific themes, designed to explore mechanisms of early nutrition programming, long-term outcomes of early

programming from infancy to adulthood and potential dietary interventions in pregnancy.

In keeping with EarlyNutrition's ethos of international collaboration, the NIGO Health protocol, funded in part by Abbott Nutrition (Spain and USA), has incorporated results from IGPOP and has been developed in unison with colleagues from IGPOP, together with principle investigators from the two recruitment sites (Granada, Spain and Munich, Germany).

NIGO Health is a prospective RCT specifically for obese pregnant women (target n=324) comparing SD-LGI nutritional supplementation plus regular dietary support against routine clinical care (habitual diet). The intervention group will receive dietary recommendations for pregnancy based on recognised national standards and instructions on how to incorporate the study beverage into these recommendations without causing an extra calorie burden.

Primary outcome is maternal blood glucose AUC at 28 weeks' following a 75gram OGTT and secondary outcomes include neonatal body composition within 72 hours of birth and maternal FBG at 36 weeks' gestation. Maternal blood will be collected at intervals in addition to cord blood, for the purposes of mechanistic and epigenetic studies.

10 CLINICAL RELEVANCE AND RESEARCH ARISING FROM THIS THESIS

With 17-40% of pregnancies considered unplanned (Wellings, Jones et al. 2013) and poor compliance with existing preconception services (Glinianaia, Tennant et al. 2014), focussing on early pregnancy in our contemporary obese antenatal population is equally important as directing resources on the preconception period only.

10.1 UPBEAT Biomarkers and Prediction of GDM

Understanding the role of adipose tissue in IR and identifying specific adipokines remains central to on-going research. Fetal over growth is only explained in part by the Pedersen hypothesis. The pro-inflammatory cascade, mediated by macrophage secretion of adipocytokines and insulin mediated lipolysis are perhaps more relevant in an obese population. The range examined in this thesis is comprehensive in comparison to similar studies and results are consistent with data supporting the role of adiponectin but thus far studies remain underpowered. The same biochemical analysis will be repeated on the full UPBEAT cohort (n=1555, GDM n=172 standard care group and n=160 intervention group, p=0.68) with further collaborations planned with the similar populations in the LIMIT and ROLO trials to generate meta-analyses (Walsh, McGowan et al. 2012, Dodd 2014).

We did not demonstrate a difference in the concentration of free fatty acids between women with and without GDM nor any clear influence of the intervention but since FFAs are directly influenced by diet, larger studies are required to further the results of the small pilot studies in obese pregnant women previously discussed (Harmon, Gerard et al. 2011, Hernandez, Van Pelt et al. 2014).

Evidence to suggest that adiponectin may be a modifiable target following a lifestyle intervention in various obese non-pregnant groups ranging from those with significant CV risk (Navaneethan, Fealy et al. 2015) to adolescents are emerging (Rambhojan, Bouaziz-Amar et al. 2015). Increased concentrations of adiponectin associated with reductions in measures of IR and CV risk have typically been in the

context of weight reduction therefore studies in pregnancy with progressive weight gain are still needed.

Lifestyle and dietary interventions may not have significantly reduced GDM incidence or adverse obstetric outcomes in obese women thus far but multiple positive maternal outcomes have been reported.

Universal GDM screening for all obese women recommend by NICE, in combination with new OGTT diagnostic glucose thresholds (NICE 2015) are not currently adopted by all UK obstetric centres due to pressures on clinical service and cost implications particularly in urban areas with high obesity rates. A robust GDM prediction model would therefore serve to better identify those women requiring an OGTT. At present a range of screening strategies are used for example across King's Health Partners where a 2-step approach measuring a random venous glucose concentration prior to OGTT is followed. Conversely the ability to predict those women with a low probability of developing GDM would reduce the burden of additional OGTTs performed and help to streamline the diagnostic pathway. Utilising prediction models, in a similar to those routinely used in cardiovascular medicine would facilitate delivery of interventions to those who would benefit most and if delivered in groups, minimise the impact on current clinical services.

Motivation to improve lifestyle factors during pregnancy is invariably high. As such, more accurate prediction of health risks in and beyond pregnancy would enable women to make informed decisions for their future health. It is not known whether maternal benefits of these relatively short duration interventions (GWG reduction, increased physical activity and dietary modifications) (Dodd, Cramp et al. 2014, Poston, Bell et al. 2015) extend beyond pregnancy or influence later health and further longitudinal assessment of these study cohorts would be useful. This would add to current knowledge that intensive exercise and metformin reduce progression to T2DM in women with previous GDM by up to 40% (Aroda, Christophi et al. 2015).

Incorporating recommendations of the TRIPOD statement, the GDM prediction model will be redeveloped and repeated on the now complete UPBEAT study of

1555 women. External validation will then need to be performed to ensure reproducibility of the model before widespread clinical usefulness can be evaluated.

10.2 Improving Glycaemic Profiles in Obese Pregnant Women

Medical nutrition therapy centred on global CHO reduction to limit postprandial glycaemia (PPG) and attenuate the effects of glucose-mediated pregnancy complications has been the initial approach to GDM management for over 20 years (Metzger 1991). At present, wide variations in dietary advice exist without international consensus. Nonetheless conventional practice to restrict CHO composition to 30-40%, from an average of 50-60%, is considered optimal for controlling glycaemia and limiting excessive weight gain although this has typically resulted in greater fat consumption e.g. CHO 40%, fat 45%, protein 15% (2013). This approach has remained relatively unchallenged but as evidence gathers in favour of modifying CHO composition/quality as opposed to quantity, to limit potential adverse effects of increased maternal fat intake, the focus is beginning to shift (Porcellati, Lucidi et al. 2013).

More recently, outwith of pregnancy, the American Heart Association (AHA) and American College of Cardiology (ACC) have published a joint statement recommending a diet of similar macronutrient composition to that followed in the IGPOP study (CHO 60.7% and fat 20.8%) for reducing cardiovascular risk (CHO 55-59% and fat 26-27%) (Eckel, Jakicic et al. 2014).

Traditional approaches of in managing hyperglycaemia pregnancy with CHO reduction typically result in greater total fat consumption. With significant and increasing numbers of obese pregnant women this approach may warrant review to examine CHO composition rather than total composition to avoid any additional caloric burden to this high-risk group. Further evaluation of the nutritional supplement in an adequately powered RCT has been established.

11 OUTPUT FROM THIS THESIS

11.1 Publications

A summary of publications to date is given below:

Textbook of Diabetes and Pregnancy, 3rd Edition, CRC Press: Boca Raton, 2015
Hod M, Jovanovic L, Di Renzo GC, de Levia A, Langer O (eds)
Interventions to improve pregnancy outcomes in obese pregnancy; implications for mother and child. *R Maitland* and L Poston

Prediction of Gestational Diabetes in Obese Pregnant Women from the UK Pregnancies Better Eating and Activity (UPBEAT) Pilot Trial
RA Maitland, PT Seed, AL Briley, M Homsy, S Thomas, D Pasupathy, SC Robson, SM Nelson, N Sattar, L Poston. On behalf of the UPBEAT trial consortium
Diabetic Medicine. 2014; 31 (8):963—70

A Slow-Digesting, Low-Glycaemic Index Nutritional Supplement (SD-LGI) in combination with a controlled diet improves glucose tolerance in obese pregnant women without Gestational Diabetes
Rahat Maitland, Nashita Patel, Suzanne Barr, Christina Sherry, Barbara Marriage, Paul Seed, Llenalia Garcia Fernandez, Jose Lopez, Helen Murphy, Ricardo Rueda, Lucilla Poston
Manuscript in preparation for submission to Diabetes Care

11.2 Presentations

Improvements to biomarkers associated with the metabolic syndrome in obese pregnant women following a lifestyle and dietary intervention: results from the UK Pregnancies Better Eating and Activity Trial (UPBEAT) pilot
R Maitland, A Briley, P Seed, N Sattar, S Barr and L Poston on behalf of the UPBEAT trial consortium
The 8th International Diabetes in Pregnancy Symposium, Berlin, April 2015 (poster)

A pilot study to evaluate the effects of a dietary supplement with slow digesting-low GI (SD-LGI) carbohydrates in obese pregnant women using continuous glucose monitoring

Maitland RA, Patel N, Sherry C, Marriage B, Barr S, Lopez JM, Murphy H, Thomas S, Fernández LG, Rueda R, Poston L
The Power of Programming, Munich, March 2014 (oral)

A pilot study of a low glycemic index dietary supplement for obese pregnancies.

R Maitland, N Patel, S Barr, C Sherry, B Marriage, JM López-Pedrosa, H Murphy, R Rueda, L Poston
Diabetes Pregnancy Study Group (DPSG) annual meeting, Malta, October 2013 (oral poster)

Prediction of gestational diabetes (GDM) in obese pregnant women (UPBEAT Study)

RA Maitland, N Sattar, P Seed, A Briley, S Thomas, D Pasupathy, L Poston
Diabetes UK Annual Professional Conference, March 2013 (oral)

Incidence of gestational diabetes (GDM) in an obese population using the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria in the UK Pregnancies Better Eating and Activity Trial (UPBEAT) pilot study

RA Maitland, S Barr, A Briley, P Seed, L Poston
Diabetes UK Annual Professional Conference, March 2012 (poster)

12 APPENDIX 1: IGPOP SUPPLEMENTARY DOCUMENTS

Supporting documents granted ethical approval for use in the IGPOP study, are included in this chapter for review. A hard copy of the stage 2 participants handbook is also provided with the thesis.

The following documents are summarised below:

1. IGPOP Stage 1 and 2 participants' preparation sheet
2. IGPOP Stage 1 participants' information sheet
3. IGPOP Stage 1B participants' information sheet
4. IGPOP Stage 2 participants' information sheet
5. IGPOP Stage 2 meal choices with detailed dietary macronutrient composition

12.1 IGPOP Study Preparation Sheet

Instructions for the night before the study visit (for all four visits):

- Do not eat or drink anything **after 10pm** the night before the study visit (water is allowed at anytime including the morning of the study visit).
- Prior to fasting have a standard meal containing between **30-50 grams** of carbohydrate
    - Please see the instructions and examples provided overleaf.
- Avoid heavy intensity physical activity/ exercise the evening before the visit.
- Avoid alcohol and caffeine the evening before the visit.

Please remember to bring the following with you to the research facility:

- Taxi receipts if applicable (however the majority of taxi journeys will be arranged through an Addison Lee account).
- Maternity notes (if applicable) – **please bring these to every visit.**
- You may wish to bring a few layers of clothing as the air conditioning in the research facility can be quite cold.
- There will be a waiting period between blood samples; therefore you may wish to bring some reading material with you. There is also wifi if you wish to bring a laptop or tablet.

Directions to the research centre:

Clinical Research Facility, 4th floor, North Wing, St Thomas' hospital

Instructions for the evening meal: In order to provide an accurate assessment of your glucose (blood sugar) levels on the study day, it is important to standardise the previous evening meal to **include between 30-50 grams of carbohydrate**. Meal suggestions are provided in table 1, however you can substitute for others as listed in table 2, provided the total does not exceed 50g carbohydrate. The most accurate way to know how much carbohydrate you are eating is by weighing the food.

A few points to consider:

- If you are planning on eating pre-packaged food, use the **'nutritional information'** table on the back which will tell you the carbohydrate content, look for the **'total carbohydrate'** rather than the 'of which sugars'. Remember to look at the **'per pack/ per portion'** carbohydrate values as these will also be listed as per 100g.
- If you leave any food or share any pre-packed food, please factor this in to your estimation of carbohydrate intake.
- Starchy and sweet foods are the main source of carbohydrates, **however** fruits, vegetables, fruit juices and milk also containing carbohydrates as well as sauces such as gravy, ketchup and pickles in smaller amounts.
- Meats, fish and fats such as butter and oils do not contain carbohydrates, therefore can be eaten freely.

Table 1. Example meals:

Meal	Carbohydrate content
Homemade meal examples:	
Chicken and curry sauce (150g) with small average portion rice (100g – approximately 3 tablespoons)	4grams
Roast beef / chicken + 3 small roast potatoes , 2 tablespoons peas + gravy	40grams
2 tablespoons cous cous + meat /fish + salad + 1 yoghurt (200g)	45grams
Medium jacket potato with cheese or tuna and side salad.	50grams
Ready meals examples:	
Sainsbury's Fish Pie, Be Good To Yourself 450g	41grams per pack
Sainsbury's Chilli Con Carne, Be Good To Yourself 400g	50grams per pack
ASDA Good For You Chicken Tikka Masala & Pilau Rice	49grams per pack
ASDA Good For You Tomato, Chicken & Basil Pasta (350g)	46grams per pack
TESCO Light choices chicken and vegetable pasta 400g	50grams per pack
TESCO Light choices white fish &steamed potates 400grams (plus x1 200g yogurt)	30grams per pack+15grams for yogurt=45 grams

Contact details: Dr Rahat Maitland, Diabetes Research Doctor Tel: 07974940221

Table 2. Carbohydrate contents of common foods

Food	Portion size	Carbohydrate content (grams)
White or Wholemeal bread	1 thick slice (large loaf – 45g) 1 medium slice (large loaf – 35g) 1 thin slice (small loaf – 25g)	20 15 10
Granary Bread	1 medium slice (35g)	25
Pitta Bread	1 standard (75g)	45
Burger Roll	1 (50g)	25
All Rice – white, brown, basmati	Small portion (100g) Medium portion (180g) 1 heaped tablespoon (40g)	30 50 10
White & Wholemeal Spaghetti Pasta (any shape)	Small portion (150g) Medium portion (230g) 1 heaped tablespoon (30g)	30 45 5
Macaroni Cheese	Average portion (220g)	30
Couscous	1 tablespoon (33g)	15
Tinned Spaghetti	Small tin (215g)	25
Baked/ kidney/ butter beans	3 tablespoons (120g)	20
New potatoes, boiled	1 average (40g)	5
Chips	Per 5 chips (50g)	10
Roast Potato	1 small (50g)	10
Mashed Potatoes	1 Scoop (60g) 2 tablespoons (90g)	10 15
Peas	2 tablespoons (60g) Canned, 2 tablespoons (60g)	5 10
Sweetcorn canned	2 tablespoons	15
Yorkshire Pudding	1 average (80g)	20

Pizza (cheese & tomato) - 12"	1/2 thin crust (150g)	50
Thick Sausages/Fish fingers	2 (40-50g)	10
Breaded fish	1 fillet (150g)	20
Breaded chicken	1 individual (170g)	15
Stuffing	1 tbsp (30g)	5
Fishcake	1 (50g)	20
Sugar or Glucose	2 level teaspoons (8g)	8
Honey, Jam or Syrup	2 level teaspoons(8g)	8
Fizzy Drinks (Not Diet) e.g. Coca Cola	100mls/4floz	5
Orange Juice	100mls/4floz	10
Fresh milk: Skimmed & Full Cream, Semi-skimmed	200mls (1/3 pint)	10
Natural Yoghurt	Small Carton (125g)	10
Low fat Yoghurt	Small Carton (125g)	20
Diet Yoghurt e.g. Shape, Muller Light	Small Carton (125g) 1 tub (200g)	10 15

12.2 IGPOP Stage 1A Patient Information Sheet

Guy's and St Thomas' Hospital NHS
NHS Trust

Participant Information Sheet

You are being invited to take part in a research study. Before you decide to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and do not hesitate to ask us if there is anything that is not clear or if you would like more information. Feel free to talk to others about the study and take time to decide whether or not you wish to take part. This research contributes towards a medical doctoral (MD) educational qualification.

PART 1

Title of project: Blood sugar profiles following consumption of low glycaemic index nutritional products.

1. **What is the purpose of the study?**

This study will test the blood sugar response to drinks with a different glycaemic index values. The glycaemic index of a food is the response of blood sugar to that particular food or drink, compared with an equivalent amount of a standard food (glucose). Foods or drinks with a high glycaemic index affect blood sugar levels quickly and foods or drinks with a low glycaemic index affect blood sugar levels slowly. We plan to test the glycaemic index of three different drinks when compared to a drink with the same amount of carbohydrate (a control drink). Once the blood glucose response to these drinks has been determined they will be used in a second study, which you are not required to take part in. This research is sponsored by a commercial organisation and will be carried out in a research healthcare setting by healthcare professionals.

2. Why have I been chosen?

You have been selected because you are a female aged 18-40 years and fit into one of the groups of women below based on weight and pregnancy status.

We will be testing the nutritional supplement in 4 different groups of women; pregnant and non pregnant and lean and obese (as defined by your weight for height) to see if this makes a difference to how the supplements affect blood sugar levels.

5. Lean non-pregnant (BMI 18.5-25kg/m^2)
6. Obese non-pregnant (BMI ≥30kg/m^2)
7. Lean pregnant (BMI 18.5-25kg/m^2)
8. Obese pregnant* (BMI ≥30kg/m^2)

The pregnant women will be between 24-28 weeks gestation and have single pregnancies only. You would not be able to take part if you have diabetes that was diagnosed either before or during your pregnancy. The study would require four morning visits to the hospital and each time you would need to come fasted.

3. What is being tested?

We will be testing three different drinks which vary in their glycaemic index, plus a control drink (4 in total). These drinks contain only nutrients which are part of our normal diets (fats, sugars, starches, protein and vitamins and minerals), and the three drinks will vary in the proportion of these nutrients. The drinks do not contain anything that is not considered food.

4. What will happen to me if I take part?

It is up to you to decide of you want to take part. The data and samples collected for the study are not part of any routine care you may be receiving. If you do decide to take part you will be given this information sheet to keep and will be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of any health care you receive. If you withdraw from the study, we will use any data collected up to the point of your withdrawal.

If you decide to take part you will be asked to attend the research centre on four separate occasions over a period of seven or eight days at two day intervals (e.g. every other day),

Once we have received your signed consent form we will send you a pre-appointment study pack prior to your first appointment at the study centre. This contains a medical screening questionnaire to confirm your eligibility to take part and detailed instructions on what to do the night before the study. Complete the medical screening questionnaire and return to the research team in the prepaid envelope as soon as possible. A member of the research team will contact you on receipt of your form to confirm that you are eligible to be included in the study and to arrange a date for you to start the study.

You will need to provide written consent to participate and to complete a medical screening questionnaire. You need to be able to visit the research centre in the morning fasted on four

occasions for approximately 4.5 hours (days 1, 3, 5, 7 and 8). A number of measurements will be taken including blood samples as detailed in the box above. We appreciate how busy you are and are grateful for the time given to come to the hospital. As you will need to come to the hospital fasted on every occasion we will arrange complementary taxi transport. Compensation will be provided for the inconvenience of taking part in the form of £150 worth of shopping vouchers.

1. What are the possible disadvantages and risks of taking part?

The drawing of blood may include some discomfort when the needle enters the finger. If any of your blood sugar measurements are outside of the normal range your GP will be informed in writing to enable these to be retested and managed as appropriate.

2. What are the possible benefits of taking part?

This study is unlikely to have any personal benefit to you. As a result of participating in the study you can receive a summary of your body measurements (weight, body mass index, blood pressure and blood sugar level).

Both principle investigators are registered health professionals and therefore have the necessary expertise to undertake the research project described

At the research centre the following procedure will be followed:

Evening before the appointment:

Consume a standard evening meal (as described by the research team) and then, after 8 pm, do not eat or drink anything other than water.

Appointment day:

Do not eat or drink anything other than water until after your visit to the study centre.

Attend an early morning appointment at the study centre, (St Thomas Hospital, London). You will be required to be at the study centre for approximately 4.5 hours.

- A trained person will take small finger-prick blood samples from your finger at nine timepoints within the 4.5 hours.
- On three occasions you will be asked to consume a drink containing different proportions of nutrients which are part of our normal diets (fats, sugars, starches, protein and vitamins and minerals) so that your body's blood sugar response to these can be assessed.
- On one further occasion you will be asked to consume a control drink containing the same amount of carbohydrate so that your body's response to this can be assessed.
- A trained person will take measurements of your blood pressure, weight and height.

You will then be offered a meal and be free to leave the study centre.

3. What do I have to do?

You will need to provide written consent to participate and to complete a medical screening questionnaire. You need to be able to visit the research centre in the morning fasted on four

occasions for approximately 4.5 hours (days 1, 3, 5, 7 and 8). A number of measurements will be taken including blood samples as detailed in the box above. We appreciate how busy you are and are grateful for the time given to come to the hospital. As you will need to come to the hospital fasted on every occasion we will arrange complementary taxi transport. Compensation will be provided for the inconvenience of taking part in the form of £150 worth of shopping vouchers.

4. What are the possible disadvantages and risks of taking part?

The drawing of blood may include some discomfort when the needle enters the finger. If any of your blood sugar measurements are outside of the normal range your GP will be informed in writing to enable these to be retested and managed as appropriate.

5. What are the possible benefits of taking part?

This study is unlikely to have any personal benefit to you. As a result of participating in the study you can receive a summary of your body measurements (weight, body mass index, blood pressure and blood sugar level).

Both principle investigators are registered health professionals and therefore have the necessary expertise to undertake the research project described.

If you are interested and are considering taking part, please continue to read the additional information in Part 2 before you make a decision.

PART 2

6. What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. If you have a concern about any aspect of your participation, please raise this with Dr Rahat Maitland (Rahat Maitland, Research Diabetes Doctor) Tel: 07974940221 or 020 7188 7804 or Dr Suzanne Barr (0207 848 3360) who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do so by contacting the normal NHS complaints mechanism.

7. Will my taking part in the study be kept confidential?

Yes. All the information about your participation in this study will be kept confidential. A code number will be used to identify any information provided from questionnaires and samples. This information will be stored securely and only accessed by the named investigators. No names will be used in any reports or publications related to this work.

8. What will happen to any samples I give?

No samples are being stored, as the glucose the glucose meter will give levels from the finger prick test instantly.

9. What will happen to the results of the research?

The results will be published in a scientific journal. The results will also inform a second larger study which plans to use the drinks tested. Participants will not be identified in any reports or publications arising from the study.

Your glucose results may be used in future studies with academic and commercial partners looking at other pregnancy related problems. All such projects will have been granted ethical approval.

10. Who is organising and funding the research?

Abbott Nutrition, a commercial company, has funded this study. The midwife and research nurses working on this study have their salaries paid by this organisation. The hospital, doctors and dietitian do not receive any payment if you help with this research.

11. Who has reviewed the research?

The Riverside research ethics committee has reviewed this study.

If you agree to take part in the study you will keep a copy of this information sheet and a signed consent form.

12. What do I do if I have further questions or want to take part?

For further information please contact:

Suzanne Barr, Research Dietician

Tel: 020 7848 3360

Rahat Maitland, Diabetes Research Doctor

Tel: 07974940221 or 020 7188 7804

Members of the Research Team:

Dr Rahat Maitland and Dr Suzanne Barr

Collaborating investigators:
Professor Lucilla Poston
Dr Ricardo Rueda
Dr Barbara Marriage
Dr Helen Murphy
Dr Jose M Lopez
Dr Stephen Thomas
Dr Christina Sherry

12.3 IGPOP Stage 1B Patient Information Sheet

Guy's and St Thomas' Hospital NHS
NHS Trust

Participant Information Sheet

You are being invited to take part in a research study. Before you decide to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and do not hesitate to ask us if there is anything that is not clear or if you would like more information. Feel free to talk to others about the study and take time to decide whether or not you wish to take part. This research contributes towards a medical doctoral (MD) educational qualification.

PART 1

Title of project: Blood sugar profiles following consumption of a low glycaemic index nutritional product.

1. **What is the purpose of the study?**

This study will test the blood sugar response to a drink with a low glycaemic index value. The glycaemic index of a food is the response of blood sugar to that particular food or drink, compared with an equivalent amount of a standard food (glucose). Foods or drinks with a high glycaemic index affect blood sugar levels quickly and foods or drinks with a low glycaemic index affect blood sugar levels slowly. We plan to test the glycaemic index of a drink when compared to a glucose (sugar) drink. Once the glycaemic index value of this drink has been determined it will be used in a second study, which you are not required to take part in. This research is sponsored by a commercial organisation and will be carried out in a research healthcare setting by healthcare professionals.

2. **Why have I been chosen?**

You have been selected because you are a female aged 18-40 years, have a normal body mass index (normal weight for height [BMI 18.5-25kg/m^2]), are not pregnant and have no known diabetes. The study will require two morning visits to the hospital and each time you would need to come fasted.

3. **What is being tested?**

We will be testing a drink which is thought to have a low glycaemic index plus the standard glucose drink. The drink contains only nutrients which are part of our normal diets (fats, sugars, starches, protein and vitamins and minerals). The drinks do not contain anything that is not considered food.

4. **What will happen to me if I take part?**

It is up to you to decide of you want to take part. The data and samples collected for the study are not part of any routine medical care you may be receiving. If you do decide to take

part you will be given this information sheet to keep and will be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of any health care you receive. If you withdraw from the study, we will use any data collected up to the point of your withdrawal.

If you decide to take part you will be asked to attend the research centre on two separate occasions over a period of three days with a rest day in between (e.g. every other day),

Once we have received your signed consent form we will send you a pre-appointment study pack prior to your first appointment at the study centre. This contains a medical screening questionnaire to confirm your eligibility to take part and detailed instructions on what to do the night before the study. Complete the medical screening questionnaire and return to the research team in the prepaid envelope as soon as possible. A member of the research team will contact you on receipt of your form to confirm that you are eligible to be included in the study and to arrange a date for you to start the study.

At the research centre the following procedure will be followed:

Evening before the appointment:

Consume a standard evening meal (as described by the research team) and then, after 8 pm, do not eat or drink anything other than water.

Appointment day:

Do not eat or drink anything other than water until after your visit to the study centre.

Attend an early morning appointment at the study centre, (St Thomas Hospital, London). You will be required to be at the study centre for approximately 2.5 hours.

- A trained person will take small finger-prick blood samples from your finger at seven time points within the 2.5 hours.
- At one appointment you will be asked to consume a drink containing 50g glucose (sugar) so that your body's response to this can be assessed.
- On the other occasion you will be asked to consume a drink containing different proportions of nutrients which are part of our normal diets (fats, sugars, starches, protein and vitamins and minerals) so that your body's blood sugar response to this can be assessed.
- A trained person will take measurements of your blood pressure, weight and height.

You will then be offered a meal and be free to leave the study centre.

5. What do I have to do?

You will need to provide written consent to participate and to complete a medical screening questionnaire. You need to be able to visit the research centre in the morning fasted on two occasions for approximately 2.5 hours (days 1 and 3). A number of measurements will be taken including blood samples as detailed in the box above. We appreciate how busy you are

and are grateful for the time given to come to the hospital. As you will need to come to the hospital fasted on every occasion we will arrange complementary taxi transport. Compensation will be provided for the inconvenience of taking part in the form of £75 worth of shopping vouchers.

6. What are the possible disadvantages and risks of taking part?

The drawing of blood may include some discomfort when the needle enters the finger. If any of your blood sugar measurements are outside of the normal range your GP will be informed in writing to enable these to be retested and managed as appropriate.

7. What are the possible benefits of taking part?

This study is unlikely to have any personal benefit to you. As a result of participating in the study you can receive a summary of your body measurements (weight, body mass index, blood pressure and blood sugar level).

Both principle investigators are registered health professionals and therefore have the necessary expertise to undertake the research project described.

If you are interested and are considering taking part, please continue to read the additional information in Part 2 before you make a decision.

PART 2

8. What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. If you have a concern about any aspect of your participation, please raise this with Dr Rahat Maitland (Rahat Maitland, Research Diabetes Doctor) Tel: 07974940221 or 020 7188 7804 or Dr Suzanne Barr (0207 848 3360) who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do so by contacting the normal NHS complaints mechanism.

9. Will my taking part in the study be kept confidential?

Yes. All the information about your participation in this study will be kept confidential. A code number will be used to identify any information provided from questionnaires and samples. This information will be stored securely and only accessed by the named investigators. No names will be used in any reports or publications related to this work.

10. What will happen to any samples I give?

No samples are being stored as the glucose levels from the finger prick test will be given instantly by the glucose meter.

11. What will happen to the results of the research?

The results will be published in a scientific journal. The results will also inform a second larger study which plans to use the drinks tested. Participants will not be identified in any reports or publications arising from the study.

Your glucose results may be used in future studies with academic and commercial partners looking at other pregnancy related problems. All such projects will have been granted ethical approval.

12. Who is organising and funding the research?

Abbott Nutrition, a commercial company has funded this study. The midwife and research nurses working on this study have their salaries paid by this organisation. The hospital, doctors and dietitian do not receive any payment if you help with this research.

13. Who has reviewed the research?

The Riverside research ethics committee has reviewed this study.

If you agree to take part in the study you will keep a copy of this information sheet and a signed consent form.

14. What do I do if I have further questions or want to take part?

For further information please contact:

Suzanne Barr, Research Dietician

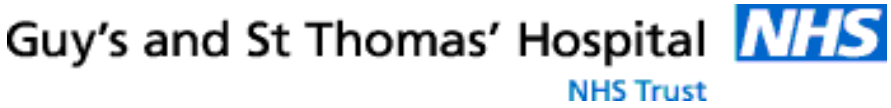
Tel: 020 7848 3360

Rahat Maitland, Diabetes Research Doctor

Tel: 07974940221 or 020 7188 7804

Member of the research team:
Dr Rahat Maitland and Dr Suzanne Barr
Collaborating investigators:
Professor Lucilla Poston
Dr Ricardo Rueda
Dr Barbara Marriage
Dr Helen Murphy
Dr Jose M Lopez
Dr Stephen Thomas
Dr Christina Sherry

12.4 IGPOP Stage 2 Participant Information Sheet



IG-POP (**I**mproving **g**lycaemic **p**rofiles in **o**bese **p**regnancies) Stage 2

Background

We all know that people are getting heavier and this can lead to many different types of health issues. Many women who are obese when they are pregnant have no problems and deliver a healthy baby. However we do know that obese women are more at risk of pregnancy related complications than women who are lean. These complications include miscarriage, high blood pressure and gestational diabetes (GDM). GDM can cause the baby to grow excessively and be larger than expected which can cause problems at delivery. We also know that pregnant women with GDM have a higher risk of developing diabetes later in life after their baby is born.

In gestational diabetes blood glucose levels (sugar) are higher than normal for pregnancy. We know that women who are heavier have higher glucose (sugar) levels than lean women, which may not be high enough to have actual gestational diabetes. Changing the diet is one of the best ways to help keep the blood sugar levels down and prevent gestational diabetes.

We want to see if a nutritional supplement made especially for obese pregnant women can help to lower glucose levels and reduce the chances of getting gestational diabetes. The nutritional supplement is already sold overseas for people who are overweight and are keen to try and lower their blood glucose level almost like spreads, which you see advertised on the TV to help people lower their cholesterol levels. This supplement has been changed slightly to provide a range of pregnancy specific vitamins and minerals.

It would be very useful to see how long this new nutritional supplement works for and how long it can help lower your blood glucose levels. To do this we can use the most up to date technology. A tiny sensor is used and the tip (less than half a cm) is put in just under the skin. This measures the glucose levels every minute without having to do lots of blood tests. We know that it is safe to use in pregnancy and it can be worn for up to 5 days and give us lots of helpful information. Once it has been put in, you can't feel it.

We are also interested in whether the emotional and physical changes that occur during pregnancy are affected by a woman's weight when she becomes pregnant. For example, research has suggested that a large proportion of pregnant women experience periods of anxiety, low mood and binge eating during pregnancy but little is known about how these are related to women's weight. This part of the study called "Investigating Attitudes to Eating in Pregnancy" will explore mental health and eating patterns in pregnancy.

Why have I been chosen?

You have been asked to take part because you are pregnant and had a body mass index (BMI) of 30 or more when you first saw your midwife or doctor.

What do I have to do if I take part?

You will be seen by a member of the research team who could be a midwife or doctor who will answer any questions you may have. You will be asked a few questions about you medical and obstetric history to make sure you can take part in the study and some questions about your current eating habits. At this time we will also show you the small glucose sensor so you can have a look to see how it works and also have a trial of wearing it. Once you have agreed to take part you will be asked to sign a consent form and be given a copy of this to keep.

The whole study lasts 7 days but you will only need to come to the hospital on 2 visits to take part. Before the actual study starts we will send you a food diary to fill in with help on how to do this which will be reviewed by a dietician. The visits will be in the morning and you will need to come to hospital fasted. You will then have your breakfast in the hospital and a mid-morning snack also. During this time blood samples will be taken at regular times but this will be through a cannula (like those used for drips) to avoid you having lots of blood tests. The glucose sensor will be fitted and we will make sure you know how to manage it. After this has been done you go can go home and we will make sure you have a contact number if you have any problems. After the weekend you will come back to the hospital fasted again. You will have your breakfast and mid-morning snack with us and we will take some blood samples again. After this you can go home. We will contact you 2 days later to arrange either removing and picking up the glucose sensor at your home or arranging a time for you to come back to the hospital to have it removed.

All blood samples will be processed and frozen. These will be barcoded and stored so no one will know the sample is yours for up to 6 years. Further studies investigating other pregnancy related problems may be carried out in the future using these samples with

academic and commercial partners but all such projects will have been granted ethical approval.

If you agree to take part in the main study we would also like to ask you take part in the separate study called "Investigating Attitudes to Eating in Pregnancy." You don't have to take part in this unless you want to.

We would like to hear about your experiences during pregnancy in a one-to-one interview. This interview will not have set questions; the interviewer will introduce some topics but then you can be in control of the discussion. These topics will be related to pregnancy, weight, eating and mood. We really want to hear your perspective. The interview will be carried out by the PhD student working on this project

We would also like to do two questionnaires with you; one includes questions about mental health and the other is about binge eating experiences.

This would happen during one of your main study appointments. The questionnaires and interview should take around 1 hour in total, and should be able to fit into breaks in the main study appointment. If you find that you have a lot to say during the interview, the session may take a little longer, but this is completely up to you and your travel expenses to get home will still be covered.

Do I have to take part?

Whether you decide to take part or not is entirely up to you. Your decision will not affect the care you receive in any way. If you agree to take part, you are free to withdraw at a later stage, without giving a reason. You can also decide whether or not you would also like to take part in the "Investigating Attitudes to Eating in Pregnancy" part of the study. If you do choose withdraw at anytime we will ask if you mind us using the results we have collected so far from you for analysis.

Will my taking part be kept confidential?

All information will be kept strictly confidential. Any information stored about you will have your name, address and other identifying details removed. All computers used will be password protected. Only people directly involved in the study will have access to the information. A letter explaining that you have taken part in this study will be inserted into your antenatal handheld notes that you keep. If any unexpected or abnormal findings are discovered as a result of the study we will refer you to the correct doctor for further follow up and let your GP know.

If you participate in the "Investigating Attitudes to Eating in Pregnancy" interview, we will ask your permission to audiotape the session. The audiotapes will be stored in a locked secure place at all times and will be destroyed at the end of the study.

If your responses during the questionnaires or interview suggest that you are suffering from an eating disorder or mental health problem, the interviewer will discuss with this you and will contact her research supervisor (a psychiatrist specialising in mental health around pregnancy). She may refer you to your GP, but only if you agree to this. The only exception to confidentiality will be if you disclose information which suggests a risk of serious harm to any person (including yourself). In this case, your GP and other clinical staff involved in your care will be informed. This would be discussed with you during your appointment.

What are the benefits of taking part?

You are unlikely to benefit personally from taking part in either study.

You may help design a nutritional supplement that would help obese women keep their blood glucose levels low and reduce their chances of getting gestational diabetes. This may help many pregnant women in the future and if we can reduce gestational diabetes this would also influence the health of their children possibly for the whole of their lives. You will receive a detailed dietary review from a research dietician who will give you some constructive feedback.

If you take part in the "Investigating Attitudes to Eating in Pregnancy" study you may help to increase healthcare professionals' understanding of what pregnant women feel about a range of topics related to weight, mental health and eating patterns.

What are the side effects of taking part?

There are no real side effects known as the nutritional supplement is already widely used in the USA and it will be changed slightly to make it more specific to the nutritional needs of pregnancy.

There are no side effects from using the small glucose sensors. Some women may find the dressing that covers the skin mildly irritating but no serious reactions have been reported.

If you take part in the "Investigating Attitudes to Eating in Pregnancy" study some of the subjects that will be discussed during the interview and parts of the questionnaires may be of a sensitive nature. However, you do not have to discuss anything which makes you feel

uncomfortable and can take a break, move on from that part of the discussion or end the interview at any time if you want to.

What happens if anything goes wrong?

In the unlikely event that you are harmed by taking part in this study no special insurance applies. However if you are harmed due to negligence normal NHS indemnity may apply but you have to pay for this action. You will be given contact details for the research team if you have any problems at any time during the study.

Regardless of whether anything goes wrong, if you wish to complain about any aspect of the way of the way you have been approached or treated during the course of the study the normal NHS complaints mechanism is available to you.

What will happen to the results of the study?

The results will be published in medical journals. Feedback on the overall findings will be available to participants following data analysis and we will also give you feedback on your food diaries by post or email one the study is finished.

Who is paying for this research?

Abbott Nutrition, a commercial company have funded this study. The midwife and research nurses working on this study have their salaries paid by this organisation. The hospital, doctors and dietician do not receive any payment if you help with this research.

This "Investigating Attitudes to Eating in Pregnancy" study is being carried out as part of a PhD Studentship funded by the Medical Research Council.

What about travel and other costs?

We appreciate how busy pregnant women are and are grateful for the time given to come to the hospital. As you will need to come fasted on every occasion to the hospital we will arrange complementary taxi transport. We are also aware that you may require some time off work and have some problems with childcare on the days you need to come to hospital. We will therefore reimburse you up to the value of £200 for any inconvenience incurred.

To show our appreciation we will invite all women and their new babies for a lunch so you can meet other women in the group, share your experiences and talk to the research team again. We would like to take a professional photograph of you and you baby at this time and will give you a copy to keep as a memento. You will also receive a new nappy bag.

We will give you a £20 shopping voucher to reimburse your time if you also take part in the "Investigating Attitudes to Eating in Pregnancy" study.

Who has reviewed this study? The Riverside research ethics committee has reviewed and approved this study.

What do I do if I have further questions or want to take part?

For further information please contact:

Rahat Maitland, Diabetes Research Doctor or Suzanne Barr, Research Dietician Tel:020 7188 7804

12.5 Meal Choices For IGPOP Stage 2

In the pre-study visit, food preferences were documented, including allergies and religious requests. In such circumstances, slight deviation from the set menus was made following review by the research dietician to ensure any changes complied with the controlled diet. Participants were offered a choice of Menu A or B for each of the study days held at the CRF.

Table 37 Alternative Meal Choices (Menu B) for CRF study days 1 and 5 (Thursday and Monday)

MENU B-Day 1 & 5	Energy (Kcal)	Total CHO (g)	Total sugars (g)	Total protein (g)	Total fat (g)	Total fibre (g)	GI
09.30 BREAKFAST							
Rice krispies (20g variety pack)	73	17	2	2	0	0	93
Intervention or control supplement	152	23	17	7	4	2	27
Meal total	**224**	**40**	**19**	**9**	**4**	**2**	
13.00 LUNCH							
Sainsbury's tomato and mozzarella pot (300g)	381	55	10	15	11	5	35
Cheddar cheese (60g)	219	0	0	14	18	0	34
Nature's Finest Tropical Fruit Salad pot (in juice) 113g	67	14	13	3	0	1	50
Meal total	**667**	**69**	**23**	**32**	**29**	**5**	
15.00 AFTERNOON							
Intervention or control supplement	152	23	17	7	4	2	27
18.30 DINNER							
Sainsbury's British Classic Shepherd's pie (450g)	461	48	2	23	19	6	35
Ambrosia Chocolate custard (150g)	171	28	22	5	4	1	38
Meal total	**632**	**76**	**24**	**27**	**23**	**6**	
20.30 SUPPER & MISC							
Philadelphia tub (35g) snack	55	1	1	3	4	0	34

Philadelphia tub (35g) snack	55	1	1	3	4	0	34
Poppy & sesame thin crackers x 4	80	10	0	2	4	1	65
Sainsbury's grape pack (80g)	53	12	12	0	0	1	46
Meal total	**243**	**25**	**16**	**8**	**12**	**2**	
Meal total excluding supplements	**1766**	**209**	**81**	**76**	**67**	**15**	
Total	1918	232	97	83	71	17	

Dietary data were generated using the WISP dietary data software

*Estimation of GI. For intervention supplement, GI given is calculated from Stage 1b IGPOP.

Table 38 Alternative Meal Choices (Menu A) for CRF study days 2 and 6 (Friday and Tuesday)

MENU A-Day 2 & 6	Energy (Kcal)	Total CHO (g)	Total sugars (g)	Total protein (g)	Total fat (g)	Total fibre (g)	GI
08.00 BREAKFAST							
Rice krispies (20g) pack)	73	17	2	2	0	0	8
Intervention or control supplement	152	23	17	7	4	2	2
Meal total	**224**	**40**	**19**	**9**	**4**	**2**	
11.00 SNACK							
Muller Amore Spanish Orange Yogurt (150g)	218	26	24	4	11	0	3
13.00 LUNCH							
John west snack pot Mediterranean style tuna salad	211	22	10	19	4	5	4
Poppy & sesame thin crackers x 4	80	10	0	2	4	1	6
Sainsbury's olive spread (15g)	80	0	0	0	9	0	
Meal total	**371**	**32**	**10**	**20**	**17**	**6**	
15.00 AFTERNOON							
Intervention or control supplement	152	23	17	7	4	2	2
18.30 DINNER							
Sainsbury's mushroom risotto (400g)	502	65	4	9	22	2	6
Yeo Valley Organic Natural Yogurt (150g)	124	10	10	7	6	0	3
Nature's Finest Tropical Fruit Salad pot (in juice) (113g)	67	14	13	3	0	1	5
Meal total	**693**	**89**	**27**	**19**	**28**	**3**	
20.30 SUPPER & MISC							
Philadelphia tub (35g) snack	55	1	1	3	4	0	3
Philadelphia tub (35g) snack	55	1	1	3	4	0	3
Poppy & sesame thin crackers x 4	80	10	0	2	4	1	6
Meal total	**190**	**12**	**3**	**7**	**12**	**1**	**13**
Meal total excluding supplements	**1478**	**173**	**59**	**55**	**61**	**12**	
Total	1848	222	99	66	75	13	

Dietary data were generated using the WISP dietary data software

*Estimation of GI. For intervention supplement, GI given is calculated from Stage 1b IGPOP.

Table 39 Alternative Meal Choices (Menu B) for CRF study days 2 and 6 (Friday and Tuesday)

MENU B-Day 2 & 6	Energy (Kcal)	Total CHO (g)	Total sugars (g)	Total protein (g)	Total fat (g)	Total fibre (g)	GI
08.00 BREAKFAST							

Cornflakes (17g from variety pack)	61	14	1	2	0	1	8
Intervention or control supplement	152	23	17	7	4	2	2
Meal total	**212**	**37**	**18**	**9**	**4**	**2**	**10**
11.00 SNACK							
Muller Amore Spanish Orange Yogurt (150g)	218	26	24	4	11	0	3
13.00 LUNCH							
Quorn lasagne (250g)	249	33	4	12	7	5	3
Poppy & sesame thin crackers x 4	80	10	0	2	4	1	6
Sainsbury's olive spread (15g)	80	0	0	0	9	0	0
Meal total	**409**	**42**	**4**	**14**	**19**	**6**	
15.00 AFTERNOON							
Intervention or control supplement	152	23	17	7	4	2	2
18.30 DINNER							
Sainsbury's chicken, bacon & mushroom pasta bake (400g)	505	56	5	26	19	5	6
Yeo Valley Organic Natural Yogurt (150g)	124	10	10	7	6	0	3
Nature's Finest Tropical Fruit Salad pot (in juice) (113g)	67	14	13	3	0	1	5
Meal total	**696**	**80**	**28**	**36**	**25**	**6**	
20.30 SUPPER & MISC							
Philadelphia tub (35g) snack	55	1	1	3	4	0	3
Philadelphia tub (35g) snack	55	1	1	3	4	0	3
Poppy & sesame thin crackers x 4	80	10	0	2	4	1	6
Meal total	**190**	**12**	**2**	**8**	**12**	**1**	
Meal total excluding supplements	**1507**	**172**	**52**	**66**	**60**	**14**	
Total	1877	221	94	77	75	16	

Dietary data were generated using the WISP dietary data software

*Estimation of GI. For intervention supplement, GI given is calculated from Stage 1b IGPOP.

13 APPENDIX 2: UPBEAT PILOT RESULTS

Table 40 Description of subjects at baseline (15^{+0}-17^{+6} weeks' gestation) by randomised treatment.

Characteristic	Control (n=58)	Intervention (n=59)	All (n=117)
Age (years)	30.8 (4.9)	30.7 (5.8)	30.8 (5.4)
18-25	11 (19.0%)	13 (22.0%)	24 (20.5%)
26-30	12 (20.7%)	16 (27.1%)	28 (23.9%)
31-40	23 (39.7%)	14 (23.7%)	37 (31.6%)
35 plus	12 (20.7%)	16 (27.1%)	28 (23.9%)
Height (m)	1.64 (0.07)	1.65 (0.07)	1.65 (0.07)
Weight (kg)	96.86 (16.82)	98.38 (12.72)	97.63 (14.85)
BMI (kg/m^2)	35.86 (4.75)	36.13 (4.80)	36.00 (4.76)
SBP (mmHg)	115.7 (7.7)	119.4 (9.4)	117.6 (8.8)
DBP (mmHg)	71.1 (6.9)	74.1 (6.7)	72.6 (6.9)
Skinfolds (mm)			
Triceps	32.4 (9.0)	33.9 (8.4)	33.1 (8.7)
Biceps	25.9 (8.5)	26. 0 (8.7)	26.0 (8.6)
Subscapular	33.3 (9.9)	34.8 (9.1)	34.0 (9.5)
Suprailiac	31.7 (8.7)	29.4 (9.3)	30.5 (9.0)
Total	90.6 (18.7)	90.1 (20.5)	90.4 (19.6)
Ethnicity			
White	38/58 (65.5%)	34/59 (57.6%)	72/117 (61.5%)
Black	18/58 (31.0%)	22/59 (37.3%)	40/117 (34.2%)
Asian	0/58 (0%)	1/59 (1.7%)	1/117 (0.9%)
Other	2/58 (3.4%)	2/29 (3.4%)	4 (3.4%)
Parity			
0	23 (39.7%)	27 (45.8%)	50(42.7%)
1	28 (48.3%)	18 (30.5%)	46 (39.3%)
2 or more	7 (12.1%)	14 (23.7%)	21 (17.9%)
Previous GDM history			
No	34 (58.6%)	28 (47.5%)	62 (53.0%)
Not applicable (para 0)	23 (39.7%)	27 (45.8%)	50 (42.7%)
Unknown	1 (1.7%)	1 (1.7%)	2 (1.7%)
Yes	0	3 (5.1%)	3 (2.6%)
History of GDM			
(multiparous only)	0/34	3/31 (9.7%)	3/65 (4.6%)
Smoking			
Never	35/58 (60.3%)	39/59 (66.1%)	74/117 (63.2%)
Ex-smoker	18/58 (31.0%)	17/59 (28.8%)	35/117 (29.9%)
Current	5/58 (8.6%)	3/59 (5.1%)	8/117 (6.8%)
Family history			
T1DM	1/58 (1.7%)	1/59 (1.7%)	2/117 (1.7%)
T2DM	16/58 (27.6%)	8/59 (24.1%)	24/114 (20.5%)

Results are given as n (%) or mean (SD)

The randomised treatment allocation is balanced by minimisation on maternal age, centre, ethnicity and parity

Table 41 Summaries of circumference measurements (cm) by randomised treatment

	Intervention (n=57)	Control (n=58)	Treatment effect: Difference in arithmetic mean (95% CI)	P*
Neck				
Baseline	37.0 (2.1)[1]	37.1 (2.9)[4]	-	-
Post-intervention	37.2 (2.3)[2]	36.9 (2.8)[5]	0.4 (-0.3 to 11)	0.2
Late gestation	37.4 (1.8)[3]	37.1 (2.6)[6]	0.4 (-0.3 to 1.1)	0.2
Waist				
Baseline	108.5 (9.2)[1]	107.5 (11.2)[4]	-	-
Post-intervention	113.6 (7.7)[2]	113.8 (10.0)[5]	-1.2 (-3.1 to 0.8)	0.2
Late gestation	117.5 (7.5)[3]	118.5 (11.3)[6]	-2.2 (-4.4 to -0.03)	0.04
Mid arm				
Baseline	37.6 (3.8)[1]	37.3 (4.1)[4]	-	-
Post-intervention	37.6 (3.6)[2]	37.2 (4.1)[5]	0.0 (-0.9 to 0.9)	1.0
Late gestation	37.9 (3.3)[3]	37.3 (3.9)[6]	0.6 (-0.3 to 1.5)	0.1
Wrist (mm)				
Baseline	172.7 (13.4)[4]	172.9 (15.2)[4]	-	-
Post-intervention	173.8 (12.3)[2]	172.9 (11.5)[5]	0.6 (-4.0 to 5.2)	0.8
Late gestation	174.9 (11.5)[3]	174.3 (11.5)[6]	1.5 (-4.4 to 7.4)	0.6
Hip				
Baseline	124.1 (11.5)[1]	121.3 (10.6)[4]	-	-
Post-intervention	124.8 (9.9)[2]	123.9 (11.2)[5]	-1.6 (-4.2 to 0.9)	0.2
Late gestation	125.5 (10.9)[3]	125.8 (10.8)[6]	-2.4 (-5.0 to 0.2)	0.1
Thigh				
Baseline	68.5 (8.7)[1]	68.6 (6.9)[4]	-	-
Post-intervention	70.6 (7.6)[2]	69.6 (7.7)[5]	0.8 (-1.3 to 2.9)	0.5
Late gestation	70.5 (7.7)[3]	71.1(6.8)[6]	0.02 (-2.3 to 2.3)	1.0

Data are presented as n=arithmetic mean (SD)

Baseline (15^{+0}-17^{+6}), Post-intervention (27^{+0}-28^{+6}), late gestation (34^{+0}-35^{+6})

[1]n=59, [2]n=53, [3]n=46, [4]n=58, [5]n=54, [6]n=44

* Effect of randomised treatment estimated by random effects Generalised Least Squares (GLS) regression clustering by patient with robust standard errors and adjustment for visit and GDM status

Table 42 Summaries of circumference measurements (cm) by GDM status

	GDM (n=29)	No GDM (n=78)	Treatment effect: Difference in arithmetic means (95% CI)	P
Neck				
Baseline	37.6 (2.2)[1]	36.7 (2.6)[3]		
Post-intervention	37.2 (2.4)[1]	37.0 (2.7)[3]		
Late gestation	37.3 (1.7)[2]	37.3 (2.4)[4]		
*Average of all visits**	37.3 (36.6 to 38.0)	37.0 (36.4 to 37.5)	0.4 (-0.5 to 1.2)	0.4
Waist				
Baseline	107.8 (7.4)[1]	107.4 (10.8)[3]		
Post-intervention	113.8 (7.4)[1]	113.7 (9.4)[3]		
Late gestation	117.4 (7.6)[2]	118.2 (10.2)[4]		
*All visits**	112.7 (110.2 to 115.2)	112.6 (110.5 to 114.8)	0.06 (-3.2 to 3.3)	1.0
Mid arm				
Baseline	37.8 (4.1)[1]	37.2 (4.0)[3]		
Post-intervention	37.6 (3.1)[1]	37.3 (4.1)[3]		
Late gestation	37.4 (3.4)[2]	37.6 (3.7)[4]		
*All visits**	37.5 (36.3 to 38.7)	37.3 (36.5 to 38.1)	0.2 (-1.3 to 1.7)	0.8

	GDM (n=29)	No GDM (n=78)	Treatment effect: Difference in arithmetic means (95% CI)	P
Wrist (mm)				
Baseline	173.9 (12.5)[1]	172.4 (15.6)[5]		
Post-intervention	174.2 (10.9)[1]	173.0 (12.3)[3]		
Late gestation	173.2 (12.8)[2]	175.1 (10.9)[4]		
*All visits**	173.8 (170.8 to 176.8)	173.2 (170.7 to 175.7)	0.6 (-3.3 to 4.5)	0.8
Hip				
Baseline	120.5 (9.2)[1]	122.9 (11.7)[3]		
Post-intervention	121.9 (9.9)[1]	125.2 (10.7)[3]		
Late gestation	120.7 (9.8)[2]	127.6 (10.6)[4]		
*All visits**	120.9 (117.7 to 124.0)	124.8 (122.4 to 127.1)	-3.9 (-7.8 to -0.01)	0.049
Thigh				
Baseline	66.4 (9.0)[1]	69.4 (7.6)[3]		
Post-intervention	69.0 (7.9)[1]	70.5 (7.5)[3]		
Late gestation	69.5 (8.2)[2]	71.3 (6.8)[4]		
*All visits**	68.2 (65.4 to 71.1)	70.2 (68.7 to 71.7)	-2.0 (-5.3 to 1.2)	0.21

Data are presented as n=mean (SD)

Baseline (16^{+0}-18^{+6}) weeks', Post-intervention (27^{+0}-28^{+6} weeks'), Late gestation (34^{+0}-35^{+6} weeks')

[1]n=29, [2]n=26, [3]n=78, [4]n=64, [5]n=77

*Comparison between GDM & non-GDM women by random effects Generalised Least Squares (GLS) regression clustering by patient with robust standard errors and adjustment for visit and randomised intervention (Arellano 1987).

14 APPENDIX 3: IGPOP BIOCHEMISTRY RESULTS

Table 43 Plasma insulin concentrations presented by visit number to the CRF measured up to 210 minutes following consumption of intervention (B) and control (D) in 16 obese pregnant women

Time (minutes)	Insulin visit 1 (mU/l)*		Insulin visit 2 (mU/l)*	
	Intervention (B)	**Control (D)**	**Intervention (B)**	**Control (D)**
0	5.9 (3.7-9.2)	5.9 (4.8-7.3)	6.0 (4.4-8.0)	5.9 (3.2-10.7)
15	13.4 (5.7-31.3)	13.2 (5.4-32.7)	14.3 (7.1-28.8)	17.0 (11.2-25.8)
30	50.8 (33.7-88.6)	34.2 (9.6-121.2)	73.8 (42.8-127.2)	55.8 (31.2-99.9)
45	54.6 (33.7-88.6)	70.9 (39.8-126.4)	74.7 (53.2-104.9)	64.4 (36.9-112.6)
60	43.2 (22.1-84.1)	55.1 (31.2-97.1)	62.4 (45.2-86.1)	42.2 (23.0-77.5)
75	16.7 (6.5-43.0)	39.4 (23.5-66.1)	39.3 (21.4-72.1)	15.4 (6.6-35.6)
90	17.8 (7.8-40.5)	32.7 (20.1-53.2)	27.6 (13.9-55.0)	21.8 (10.7-44.2)
105	12.8 (6.9 23.8)	23.8 (10.6-53.7)	30.4 (20.3-45.6)	10.8 (4.8-24.5)
120	11.7 (7.2 19.1)	18.5 (11.6-29.4)	25.6 (13.5-48.6)	10.8 (5.6-21.0)
135	6.5 (3.7-11.4)	13.3 (8.6-20.6)	16.7 (4.3-64.7)	8.9 (5.6-14.0)
150	5.8 (2.9-11.4)	11.2 (6.7-18.6)	8.4 (1.6-44.9)	8.3 (5.1-13.5)
165	5.2 (2.6-10.4)	10.7 (5.8-19.8)	9.3 (3.6-24.2)	7.6 (4.4-13.1)
180	4.3 (2.1-8.8)	6.2 (4.4-8.6)	7.3 (2.5-21.2)	5.2 (2.9-9.2)
195	3.1 (1.8-5.4)	4.9 (3.7-6.5)	6.1 (1.3-28.3)	4.9 (2.7-9.0)
210	4.0 (1.9-8.4)	3.7 (2.8-5.0)	5.2 (1.2-22.4)	4.9 (3.3-7.2)

*Data presented as geometric mean (95% CI)

Table 44 Plasma C-peptide concentrations presented by visit number to the CRF measured up to 210 minutes following consumption of intervention (B) and control (D) in 16 obese pregnant women

Time (min)	C-peptide visit 1 (pmol/l)*		C-peptide visit 2 (pmol/l)*	
	Intervention (B)	**Control (D)**	**Intervention (B)**	**Control (D)**
0	372. 8 (261.0-532.5)	361.3 (248.7-524.8)	360.6 (248.2-523.7)	452.1 (341.1-599.2)
15	573.4 (366.9-896.2)	511.5 (283.7-922.4)	493.1 (273.7-888.3)	618.6 (439.7-870.3)
30	1250.9 (895.0-1748.3)	1115.2 (575.9-2159.5)	1462.8 (950.8-2250.5)	1397.6 (957.6-2039.8)
45	1605.5 (1196.9-2153.5)	1744.0 (1063.9-2858.7)	1698.4 (1231.9-2341.4)	1764.1 (1296.4-2400.4)
60	1555.5 (1107.5-2184.8)	1678.6 (1176.1-2395.8)	1813.7 (1248.7-2634.4)	1596.8 (1161.0-2196.2)
75	1308.9 (870.2-1969.0)	1515.4 (1158.0-1983.2)	1476.7 (950.7-2293.8)	1331.1 (977.7-1812.3)
90	1112.7 (725.1-1707.5)	1383.3 (991.5-1930.1)	1259.4 (747.2-2122.8)	1268.0 (916.5-1754.1)
105	1021.5 (600.9-1736.4)	1231.1 (729.9 2076.3)	1250.6 (759.6-2058.8)	1080.9 (754.6-1548.3)
120	908.6 (574.4-1437.2)	998.1 (631.5-1577.3)	1184.6 (746.6-1879.7)	900.1 (612.0-1323.7)
135	694.9 (440.7-1095.8)	876.3 (594.0-1292.9)	1083.3 (602.2-1948.9)	788.8 (523.4-1188.7)
150	613.3 (400.1-940.1)	789.8 (552.0-1130.2)	848.9 (474.9-1517.3)	709.1 (486.1-1034.4)
165	517.4 (321.4-832.8)	730.7 (458.3-1164.9)	699.1 (338.1-1445.7)	639.6 (430.7-949.7)
180	429.4 (252.1-731.4)	532.2 (334.7-846.3)	613.3 (297.1-1265.6)	534.7 (369.7-773.3)
195	322.3 (139.7-743.6)	449.3 (299.3-674.6)	550.9 (192.1-1579.7)	501.4 (360.4-697.5)
210	366.5 (218.1-615.8)	380.0 (264.7-545.7)	475.0 (161.8-1394.2)	447.0 (324.5-615.8)

*Data presented as geometric mean (95% CI)

Table 45 Plasma NEFA concentrations presented by visit number to the CRF measured up to 210 minutes following consumption of intervention (B) and control (D) in 16 obese pregnant women

Time (min)	NEFA visit 1 (pmol/l)*				NEFA visit 2 (pmol/l)*			
	Intervention (B)		Control (D)		Intervention (B)		Control (D)	
0	0.62	(0.45-0.80)	0.47	(0.35-0.59)	0.56	(0.39-0.72)	0.67	(0.55-0.79)
60	0.27	(0.15-0.38)	0.29	(0.15-0.43)	0.34	(0.15-0.53)	0.26	(0.16-0.35)
120	0.29	(0.19-0.39)	0.14	(0.08-0.21)	0.19	(0.01-0.37)	0.17	(0.10-0.25)
180	0.63	(0.40-0.85)	0.25	(0.13-0.38)	0.23	(0.13-0.33)	0.42	(0.25-0.59)
210	0.76	(0.56-0.95)	0.39	(0.27-0.51)	0.41	(0.15-0.67)	0.65	(0.42-0.87)

*Data presented as arithmetic mean (95% CI)

Table 46 Plasma triglyceride concentrations presented by visit number to the CRF measured up 210 minutes following consumption of intervention (B) and control (D) in 16 obese pregnant women

Time (min)	Triglyceride visit 1 (mmol/l)*				Triglyceride visit 2 (mmol/l)*			
	Intervention (B)		Control (D)		Intervention (B)		Control (D)	
0	1.40	(1.06-1.74)	1.25	(0.92-1.59)	1.26	(0.94-1.59)	1.39	(1.11-1.67)
60	1.47	(1.16-1.78)	1.41	(1.04-1.77)	1.44	(0.96-1.93)	1.47	(1.19-1.75)
120	1.54	(1.15-1.93)	1.29	(0.98-1.60)	1.30	(0.83-1.78)	1.40	(1.10-1.71)
180	1.54	(1.11-1.96)	1.32	(0.95-1.70)	1.21	(0.86-1.56)	1.34	(1.04-1.64)
210	1.52	(1.14-1.91)	1.33	(0.94-1.73)	1.29	(0.81-1.77)	1.46	(1.17-1.76)

*Data presented as arithmetic mean (95% CI)

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